BIOGEOCHEMICAL PROSPECTING FOR BURIED URANIUM MINERALIZATION AND THE INVESTIGATION OF BLACK SPRUCE TREES AS A TOOL FOR BIOGEOCHEMICAL SAMPLING AND FOR ENVIRONMENTAL MONITORING OF RADIONUCLIDE CONTAMINATION

by

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Abstract

Surface geochemical methods were applied to delineate uranium (U) mineralization buried under till cover in the Jacque's Lake area, Labrador. Samples analyzed included black spruce twigs, bark, and needles; Labrador tea leaves and stems, and B-horizon soil. These results were compared to defined radiometric U/Th anomalies in the study area. Relative U enrichment followed the order: spruce twigs>bark >Labrador tea stem>spruce needles>Labrador tea leaves. Spruce twigs displayed the greatest contrasts in U contents and exhibited a significant association with the U/Th anomalies. Twig U concentrations ranged between <0.01 to 61 ppm and were highly correlated with Pb, Ag, Sb, Ca, and Be. Areas with anomalous U concentration in <250 μm sized-fraction of B-horizon soil appeared displaced east of the defined U/Th responses and the near surface projection of the buried U mineralization. There is a lack of correlation between U concentration in B-horizon soil and the defined radiometric anomalies.

The U hyperaccumulation by black spruce trees was investigated and the possible use of $^{238}U/^{235}U$ ratio as a tracer for fingerprinting the source and transport pathways of U taken up by vegetation was examined. The U accumulation property of the black spruce seedlings was evaluated by measuring the metal uptake in seedlings grown in peat and in soil treated with varying concentrations of uranyl nitrate. The bioavailability indexes of U in soil treated with 100, 250, and 400 mg U/Kg soil were estimated to be 0.21, 0.15 and 0.10 respectively. Uranium concentrations in seedling stems, the Biological Accumulation Coefficient (BAC), and Transfer Factors (TF) increased linearly with

increasing bioavailable concentration of U in the substrates. The metal accumulation in the plant tissues followed the order stem > roots > needles. Although the trend of U accumulation was comparable for plants grown in U-dosed soil versus U-dosed peat, the BAC values for plants grown in U-spiked soils were higher suggesting that metal availability was substrate specific.

The source of U taken up by the spruce seedlings was evaluated by Inductively Coupled Plasma-Quadrupole Mass Spectrometry (ICP-QMS) and Neutron Activation Analysis (NAA) analyses of $^{238}U/^{235}U$ ratios in stems of seedlings grown on substrates mounted in solutions spiked with different concentrations of DU. The $^{238}U/^{235}U$ ratio in the stems exhibited measureable deviations from the natural ratio (137.88) and ranged from 174-210 and is a consequence of the bulk uptake of U derived from the DU solutions. Elevated $^{238}U/^{235}U$ ratio in the substrates suggests upward migration of DU-enriched solution along the substrate column. The precision (1σ) for ICP-QMS determination of $^{238}U/^{235}U$ in DU-spiked substrates, and the stems of seedlings, are 0.02%-2.7% and 0.05%-1.9%, respectively.

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LIST OF ABBREVIATIONS AND SYMBOLS

BAC – Biological Accumulation Coefficient

BI – Bioavailability Index

BSE – Back Scattering Electron

CI – Confidence Interval

CMB – Central Mineral Belt

DNC – Delay Neutron Counting

FL – Florida

GPS – Global Positioning System

ICP-MS – Inductively Coupled Plasma Mass Spectroscopy

ICP-QMS – Inductively Coupled Plasma-Quadrupole Mass Spectroscopy

ICP-OES – Inductively Coupled Plasma Optical Emission Spectroscopy

MC-ICP-MS – Multi-collector Inductively Couple Plasma Mass Spectroscopy

MLA-SEM – Mineral Liberal Analysis – Scanning Electron Microprobe

MUN – Memorial University of Newfoundland

NAA – Neutron Activation Analysis

NAD – North American Datum

NE - Northeast

NH_4Ac – Ammonium acetate

NIST – National Institute of Standards and Technology

NL – Newfoundland and Labrador

ON – Ontario

RF – Radio Frequency

RSD – Relative Standard Deviation

SRM – Standard Reference Material

SW - Southwest

TF – Transfer Factor

TIMS – Thermal Ionization Mass Spectroscopy

UTM – Universal Transverse Mercator

σ – Standard Deviation

LIST OF APPENDICES

CO-AUTHORSHIP STATEMENT

The manuscript presented in chapter 2 entitled "Use of surficial geochemical methods to locate areas of buried uranium mineralization in the Jacque's Lake area of the Central Mineral Belt, Labrador, Canada" has been published in the Canadian Journal of Earth Sciences (2013), Volume 50(11), pages 1134-1146, dx:doi.org/10.1139/cjes-2013-0032.The paper is co-authored with Dr. Derek H.C. Wilton (Memorial University), Dr. Henry P. Longerich (Memorial University), Dr. Gary M. Thompson (College of the North Atlantic), and Mr. Paul McNeill (Aurora Energy Ltd). As the principal author and researcher, I was responsible for all aspects of the project including formulating specific research questions, literature review, planning and collection of field data, data analyses and interpretation, and manuscript preparation. The co-authors gave guidance in planning and collection of field data, analysis and interpretation, and corrected the written manuscript before submission. Mr. Paul McNeil assisted with the interpretation of radiometric and geologic data. The manuscript was written and submitted by me.

The manuscript presented in chapter 3 entitled "Evaluation of uranium accumulation in black spruce trees" has been published in the Research Journal of Environmental and Earth Sciences, (2013), Volume 5, Number 3, pages 113-122. The paper is co-authored with Dr. Derek H.C. Wilton (Memorial University), Dr. Henry P. Longerich (Memorial University), Dr. Gary M. Thompson (College of the North Atlantic), and Mr. Paul McNeill (Aurora Energy Ltd). As the principal author and researcher, I was responsible for all aspects of the project including formulating specific research questions; literature

review; experimental design and execution; data collection, analysis, and interpretation of data; and manuscript preparation. Dr. Derek Wilton analyzed the mineral composition of soil using the MLA-SEM technique. Co-authors gave guidance for experimental design, data collection, data reduction and interpretation, and assisted in the correction of the written manuscript. The manuscript was written and submitted by me.

The manuscript presented in chapter 4 entitled "Measurement of $^{238}U/^{235}U$ ratio in black spruce seedlings to fingerprint the source of uranium taken up by metal accumulating plants" has been submitted to the Journal of Radioanalytical and Nuclear Chemistry, manuscript #: JRNC-D-13-00762. The manuscript is co-authored with Dr. Derek H.C. Wilton (Memorial University), Dr. Gary Thompson (College of the North Atlantic) and Dr. Henry P. Longerich (Memorial University). As the primary author, I was responsible for all aspects of the project including formulating specific research questions; literature review; experimental design and execution; data collection, analysis, and interpretation of data; and manuscript preparation. Co-authors gave guidance for experimental design, data collection, data reduction and interpretation and assisted in the correction of the written manuscript. The manuscript was written and submitted by me.

Chapter 1

1-1 INTRODUCTION AND OVERVIEW

The presence of overburden cover in many regions of the world obstructs mineral exploration as well as bedrock mapping and mine development. The composition of overburden material varies in different environments and occurs in climatic settings ranging from tropical to temperate and humid to arid (Butt et al., 2000; Hamilton, 2007). Examples include vast areas in the temperate regions of the northern hemisphere overlain by an extensive cover of glacial sediments deposited during the last period of glaciation (Batterson and Taylor, 2005; McClenaghan, 1997; Kerr and Knight, 2007). At the same time, significant depths of gravel were deposited in the arid to semi-arid regions of the southwestern US and Andes (Cohen et al., 2010). Other areas with obstructive cover include northern parts of the Tasmanides in eastern Australia, which are overlain to significant depths by alluvial deposits and on-lapping Mesozoic to Tertiary sedimentary sequences (Cohen et al., 2010).

Conventional prospecting methods such as boulder tracing, trenching, stream sediment sampling, and soil geochemistry are typically used to detect mobile metallic species dispersed mechanically and/or hydromorphically from bedrock sources, but these methods have proven less effective in areas with exotic cover. To circumvent the cloaking by barren surficial cover, methods such as drift prospecting, soil-gas geochemistry, geophysical techniques, and hyperspectral surveys have been applied to locate buried mineral resources with varying degrees of success. These techniques are, however, costly, with a relatively low track record of detecting buried ore. They also have great potential to adversely impact the environment (Lintern et al., 1997; Dunn, 2007a; Reid and Hill,

2010). In particular, drilling wastes - including drilling muds, cuttings, and water and other chemicals used during drilling - contain abundant and diverse toxicants that can pose a significant risk to the environment. Radiometric surveys also have limitations, as the effective penetration depth of gamma rays is limited to the top 30 – 45 cm of the exogenous material, coupled with the additional challenge of discriminating between the heterogeneous chemical signatures of the surface soil and bedrock.

1-2 UTILITY OF PLANTS AS BIOGEOCHEMICAL SAMPLING MEDIA.

Biogeochemical analyses of vegetation have shown strong responses to buried ore in areas overlain by massive glacial sediments and alluvium, and particularly, good expressions of concealed uranium mineralization (Cannon, 1952; 61; Dunn, 1981, 1986, 2007a; Kim et al., 2013). Analysis of vegetation as a biogeochemical sampling medium is predicated on the capacity of plants to absorb labile metal species, translocate the metals, and accumulate elevated concentrations in above ground tissues. The root system of deep-rooted plants can sample many cubic meters of soil, groundwater, and bedrock structures, from which ore-related elements are taken up (Dunn, 2007a,b). Robinson (2006) established a link between the metallic element content of biomonitors and hyperaccumulator plants and the metallic composition of the underlying substrate. Consequently, the use of plant analysis in mineral exploration can provide useful expressions of buried geological features.

Plant analyses have found particular applications in the northern forest regions of North America, northern Europe, and in Australia. Analysis of U in roots of deep-rooting

Quercus and *Juniperus* trees sampled from areas overlain by exogenous glacial sediments in Utah showed significant enrichments in the vegetation with concentrations ranging from 140 to 160 ug/g (Cannon, 1952). Similarly, Cannon (1961) prospected for a sedimentary roll-front U deposit by analyzing Se in various species of *Astragalus* sampled from areas covered by Quaternary deposits in the Colorado Plateau. Selenium was used as a pathfinder element for carnotite, a potassium uranium vanadate commonly associated with sedimentary roll-front deposits in the Colorado Plateau (Dunn, 2007a). Dunn (1981) demonstrated, in studies undertaken in northern Saskatchewan and surrounding areas, that bark and the most recent years of growth of twigs of *Picea mariana* can accumulate high concentrations of uranium (>150 ppm). A regional biogeochemical survey using plant parts from balsam fir, leather leaf, Labrador tea, and bog laurel collected from areas in eastern Nova Scotia, Canada, outlined Au and other base metals - notably Cu, Pb, and Zn - contained within Paleozoic metasedimentary sequences (Rogers and Dunn, 1993). The multi-element analysis yielded up to 170 ppb Au and 72 ppm As in balsam fir twigs.

Various reconnaissance and regional biogeochemical surveys in Australia undertaken by Lintern (1997), Anand (2007), Reid et al. (2008, 2009), and Reid and Hill (2010) demonstrated the usefulness of biogeochemical procedures for delineating metalliferous zones in regolith-covered terrains. Exploration activities in this region had previously focused on sampling stream sediments, groundwater, soils/loess, and/or drilling through the blanketing exotic material to the bedrock. Cohen et al. (1999) observed that plant biogeochemistry has a better capacity to delineate broader dispersion

trains of buried mineralization than stream sediments, in a study that compared vegetation and stream sediment geochemistry over a large area of NE New South Wales. Buried geological structures hosting known gold mineralization were defined by analyzing a wide range of plant species from varying thickness of transported cover in the semi-arid Tanami Gold Province of northern Australia. In particular, soft spinifex grass discriminated a significant Au, As ±Zn, ±S, and ±Ce signature of buried gold mineralization at the Coyote and Titania prospects, whereas, snappy gum and dogwood from the Hyperion prospect were enriched in ±Au, Ce, S and Zn (Reid and Hill, 2010).

Studies undertaken in the vicinity of a low-grade, black shale-hosted, uranium deposit in the Goesan district, Korea, gave indications of buried ore in the vegetation (Kim et al., 2013). Multi-element analysis of daimyo oak leaves and red pine twigs demonstrated good associations between U enrichment in the plants and surface soils sampled from the known low-grade black shale-hosted uranium deposit. Surveys in the erosional environments in southern Morocco revealed significant associations between the geochemical responses of multi-element analyses of plant ash and the <180 μm soil fraction. The results highlighted elevated metallic element signatures in the vegetation and delineated Co-Ni mineralization and Cr carbonates more precisely than the soils (Dunn et al., 1996 *in* Anand et al, 2007)

Critics have argued that the metal contents of vegetation may not necessarily reflect substrate composition, as anthropogenic contamination could influence plant metal concentrations through the wet and dry deposition of atmospheric particulates and aerosols in addition to contaminants taken up from soil and groundwater sources.

Consequently, anthropogenic metal contaminations could create “false” surface biogeochemical anomalies that do not reflect the buried geological features. This is particularly true for plants sampled from areas proximally impacted by anthropogenic metal contamination. However, simply washing plant samples prior to analysis has been demonstrated to significantly eliminate particulates and aerosols from atmospheric fallout (Dunn, 2007a; Aksoy and Demirezen, 2006). Other perceived limitations relate to the complexities of comparing biogeochemical data from different plant tissues and/or species sampled in areas with irregular distributions of species of interest (Dunn, 2007b).

The demonstrated merits of the biogeochemical technique include a closer association between biogeochemical signatures and zones of bedrock mineralization, relative to soil/till surveys in covered terrains (Cannon, 1961; Dunn, 1981, 2007a; Reid et al. 2008, 2009; and Reid and Hill, 2010). Compared with soil, plants have the ability to sample material from greater depths and a wider area through plant root systems. Other advantages of biogeochemical exploration are the need for only a small number of samples over large survey areas, less time consumed during sampling programs, low environmental impact, and zero remediation costs compared with alternatives such as trenching, channeling, and drilling (Hulme and Hill, 2003; Dunn, 2007a).

1-2-1 BLACK SPRUCE

Black spruce (*Picea mariana*) is one of the dominant coniferous trees, with wide distribution, in the northern boreal forests of North America and northern Europe and

Asia. This evergreen conifer thrives in cool uplands, especially in poorly drained, acidic peat bogs, but is also very common along streams and the edges of swamps. The pyramid-shaped tree is small to medium-sized with an extensive lateral root system. Sinker roots can extend to depths of up to 15 m in mature trees (Ryan, 1998; Dunn, 2007a; Aspandia et al., 2008). The lateral roots of mature trees can extend at least 5 m horizontally (Ryan, 1998).

An important characteristic of black spruce as a biogeochemical sampling medium is its ability to accumulate significant concentrations of metallic elements in its tissues (Dunn, 1981, 1986, 2007). Uranium concentrations of up to 1260 ppm were detected in ash of twigs collected from the eastern margins of the Athabasca sandstone basin, and a maximum concentration of 2200 ppm U was detected in twigs of trees sampled from the vicinity of a tailings pond in areas adjacent to the Rabbit Lake open pit operations in northern Saskatchewan (Dunn, 2007). Elsewhere, Dion et al. (1993) used black spruce trees as bioindicators of anthropogenic Al contamination in the vicinity of the Alcan aluminum refinery in Jonquiere, Quebec. While there are accounts of metal accumulation by black spruce trees, there are no firmly established laboratory studies to evaluate the metal accumulation ratios.

1-3 OBJECTIVES OF THE STUDY

The ultimate goal of this research was to examine the potential for using a dominant boreal forest plant, black spruce, as a useful biogeochemical sampling medium,

and as an environmental monitoring tool in glaciated terrains of North America. The research involved two main components, field studies and the experimental investigation of U hyperaccumulation by black spruce trees. The first component, which was a case study, focused on the sampling and analysis of black spruce tissues and Labrador tea, the dominant flora over a known uranium deposit at the Jacque's Lake prospect, Central Mineral Belt (CMB), Canada. The multi-facetted approach for outlining buried U mineralization involved detailed examination of the biogeochemistry of the plants (i.e. bark and twigs of black spruce, and the stem and leaves of Labrador tea) and soil geochemistry utilizing aqua regia digestion of <250 μm sized-fraction of B-horizon soil. The plant biogeochemistry, soil geochemistry, and the U/Th responses from an airborne radiometric survey undertaken in the Jacque's Lake area were compared to delineate surface signatures of the concealed bedrock mineralization.

The second part of the study focused on the evaluation of the U-accumulation potential of black spruce trees and substrate control on the metal uptake. The biological accumulation coefficient and transfer factor for U uptake and translocation in black spruce trees were determined as a measure of U accumulation in the trees. The thesis also explored the possibility of measuring U isotope ratios in black spruce seedlings as tracers of the source of U taken up by the metal accumulating plants and to utilize the $^{238}U/^{235}U$ ratio as an indication of the incidence of radionuclide contamination in geological, environmental, and groundwater systems with which the plants interact. This is based on the assumption of an invariant isotopic ratio between the two major isotopes, ^{238}U ($t_{1/2}$ = 4.5 Ga) and ^{235}U ($t_{1/2}$ = 703 Ma) in natural U, except for the old natural reactor at Oklo,

Gabon (Stirling et al., 2007; Weyer et al., 2008). This advancement will aid in discriminating "false" uranium anomalies derived from radionuclide contamination in the biogeochemical prospecting for ore.

1-3-1 GLACIATION AND GLACIAL SEDIMENTS IN LABRADOR

As in other parts of North America, the Laurentide Ice Sheet was the last glacial event in Atlantic Canada (Batterson and Liverman, 2001). In Labrador, the ice sheets covered both low lying ground and all the high coastal summits, and extended seaward to the edge of the continental shelf (Ives, 1978). Glaciers reached the eastern coast through major valleys that cut through mountain ranges. End moraines and kames marked the edges of the advancing ice sheets. Evidence from directional flow indicators in the form of striae suggests three iceflow events in the Labrador area (Batterson and Liverman, 2001; Batterson and Taylor, 2005). McClenaghan (1997) reported, in a study undertaken on the central and eastern coast of Labrador, that earlier striae were always preserved in the lee of later events and that crossing striae were rarely observed. The initial advance, as preserved in the lee of later events, showed movement towards the northeast (045°). The consistency of this trend throughout the area suggests that it was a major event. The second flow, according to Batterson and Taylor (2005), created a broad travel path extending from the east at (090°) in southern Labrador to east-southeast (114°) in the north.

The moving ice abraded, striated, and plucked bedrock. The debris was deposited long distances from the source outcrop. Glacial debris composition is commonly

influenced by the lithology of the rock that was abraded, the nature and distance of sediment transport, and the mode of sediment deposition (Flint and Sharp, 1971; McClenaghan et al., 1997). The deposited material, typically 1–10 m thick, ranges from thin, freshly abraded, rock debris deposited near the source to thick, largely reworked material transported further from the source. The fine-fraction of the debris was carried farther and was deposited as silt and clay in glaciolacustrine environments (McClenaghan et al., 1997).

Till, the Quaternary material produced through the bulldozing and the homogenizing action of the ice during glaciation, is a poorly sorted mixture of rocks and mineral fragments - from bolder-size to clay-size sediments deposited - onto the bedrock or released by melting from the ice. The mineral composition and texture of the tills are respectively dependent on the lithology of the source rock and the reworked sediments.

1-4 ROLE OF VEGETATION IN THE CREATION OF SURFACE GEOCHEMICAL RESPONSES AND THE DETERMINATION OF BURIED GEOLOGICAL FEATURES IN AREAS OF TRANSPORTED COVER.

There has been great interest in examining the processes of metal mobilization, upward migration, and the creation of surface geochemical anomalies in exotic cover. Mechanisms, including mechanical transport by ice, water, and wind; hydromorphic dispersion by diffusion, advection, convection, capillary action, and electro-migration (Govett and Atherden, 1987; Hamilton 1998, 2007); gaseous transport via diffusion; barometric pumping; and thermal convection have been variously demonstrated to have

been responsible for the upward dispersion of elements from buried ore (Aspandia, 2008; Ma and Rate, 2009). Biological processes involving bioturbation by biota (Butt et al., 2000; Aspandia, 2008; Ma and Rate, 2009), and the hydraulic lift and uptake of labile species from buried ore through plant root systems (Aspandia, 2008) have also been suggested as viable metal transport mechanisms.

Plants remain central to biogeochemical cycling of energy and matter (e.g. metallic elements) at the interface of the biosphere, atmosphere, and geosphere. Vegetation cycling as a mechanism for transferring metallic elements from buried ore to near surface soils appears plausible due to water and nutrient movement triggered by water potential differences between leaves and roots (Aspandia, 2008). Additionally, metals stored in plant tissues get recycled into surface soils during litter decomposition and/or released into the atmosphere through phytovolatilization. Once released into the atmosphere, the volatile elements, in addition to other suspended particulates, are recycled back into the surface soils through wet and dry deposition.

1-4-1 HYDRAULIC LIFT

The mechanism of hydraulic lift involves the movement of water from a relatively moist, high water potential soil layer to dry, low water potential soil layers using plant root systems (Caldwell et al., 1998; Aspandia, 2008). The root system generally captures, stores, and/or releases water in response to environmental and physiological stimuli and in so doing alters the chemistry of the surface soil (Burgess et al., 1998). Aspandia et al. (2008) explained that plant uptake of water along a water potential gradient occurs on a

diurnal basis and is triggered by differences in the rate of transpiration between day and night, and influences the rate of water usage by the plants. At night, water taken up by plant roots along with accompanying nutrients and trace metals is released into the drier surface soil layer due to the reduced transpiration stream. Accordingly, the hydraulic lift of mineral-rich groundwater from the subsurface to the surface over long periods of time coupled with the decomposition of litter can create measureable concentrations of ore-related elements in surface soil.

1-4-2 BIOTURBATION BY PLANTS

Bioturbation by plants involves both direct movement of soil particles via plant root growth and tree-throw, and indirectly through the creation of conduits in the soil mantle for the transfer of labile metallic species from depth (Kaste et al., 2007; Ma and Rate, 2009). Pore spaces created during root growth, and perhaps tree-throw, serve as excellent pathways for the vertical migration of mobile metallic species through hydromorphic and gaseous processes.

Further, the decomposition and erosion of soils otherwise bound to root wad, and the chemical weathering of rocks transported from depth during tree-throw, can create surface geochemical haloes of the underlying mineralization. Kast et al. (2007) documented bioturbation as one of the dominant sediment transport mechanism on a forested landscape in the Bega Valley, southeastern Australia, and in grasslands of the Marin County, California, USA.

1-4-3 PLANT ROOT SYSTEM AND ROOT DEPTH

Plants have evolved root development strategies to withstand stress and foster the uptake of nutrients. Physiological and chemical modifications in root architecture, such as increased root hair length and density, the growth of special roots, and the release of exudates, facilitate the solubility of otherwise non-bioavailable metal ions and metal complexes (Ma and Rate, 2009). Rooting depth enhances the amount of water and nutrient (essential and nonessential) uptake by plants. Indeed, studies by Stone and Kalisz (1991) demonstrated the capacity of plant roots to penetrate into deeper overburden and groundwater systems to absorb plant nutrients and related labile species. Elements taken up through roots are eventually returned to the soil during litter decay, causing natural geochemical recycling, and the creation of surface geochemical signatures of the underlying bedrock. The association of deep roots and element pathways in a 40 m deep bauxite was established in a study of Jarrah forests in SW Australia. The deep penetrating roots facilitate the upward migration of Al and Fe through conduits and channels created in the overburden (Dell et al., 1983).

Poorter and Nagel (2000) observed higher biomass allocation to roots in a study of biomass distribution of plants between the roots, stems, and leaves of plants in nutrient limiting environments. Their study established that plants in nutrient-stressed environments tend to penetrate deeper and have extensive subsurface networks that allow greater access to nutrient sources and the uptake of metallic elements.

Seasonal variations were reported in the sources of metals taken up by deep-rooted plants in semi-arid environments. In particular, Pate et al., (1998) reported the

selective uptake of water from surface soils during the wet season and from groundwater sources during the dry season. The efflux of metals from plant tissues into the rhizosphere environment during the dry season contributes to the geochemical cycling of elements from depth (Greger, 1999).

Plant roots play active roles in the solubility of metal cations and complexes bound to mineral phases. Organic chemicals, e.g., mucilage, exudates, and lysates released by plants roots, induce microbiological and biochemical reactions in the rhizosphere environment. The released organic acids induce changes in rhizosphere pH, such that the pH in the immediate rhizosphere environment may differ up to 2.5 pH units from the pH of the bulk soil solution (Romheld et al., 1984). The colonization of the rhizoplane of plant root system by mycorrhizae fungi extends the effective root length by several 100 fold, thereby increasing the surface area over which essential and non-essential elements and water are taken up (Sylvia, 2005).

1-5 THESIS STRUCTURE

Chapter one provides a brief overview of mineral prospecting in regions overlain by exogenous cover with special emphasis on areas covered by glacial sediments and also highlights the challenges associated with mineral prospecting in such terrains. The history of glaciation and glacial movement in central to northeastern Labrador is also reviewed. This section also includes a concise review of the global application of plant analyses as complementary tools for defining buried mineralization in areas overlain by significant

depths of transported overburden. Following the introduction, the thesis is divided into two main sections.

The first component, presented in Chapter 2, focuses on the application of a variety of biogeochemical and soil geochemical methods to delineate areas with anomalous U concentrations in the Jacque's Lake area of the Central Mineral Belt, Labrador. The potential for using the dominant vegetation (i.e., coniferous black spruce and Labrador tea) as biogeochemical sampling media and soil geochemical methods to locate target uranium mineralization concealed under till overburden is explored. In particular, black spruce twigs and stem barks, Labrador tea leaves and stems, and B-horizon soil were analyzed to determine if the surficial samples contained detectable uranium or related pathfinder element signatures that define the buried geological features. Data obtained from biogeochemical and soil geochemical analyses are compared with U/Th responses from an airborne radiometric in the study area to establish any associations between the surface signatures of the buried mineralization and the radiometric (U/Th) responses.

The search for mineral resources at the CMB has in the past relied on prospecting methods such as trenching, boulder tracing, and geophysical techniques to detect buried mineralization (Cunningham-Dunlop et al., 2005). These methods have been demonstrated not only to be less effective in areas of exotic glacial drift, but also these methods are relatively costly. The analysis of vegetation is, therefore, complementary to and can greatly overcome the drawbacks associated with, geophysical techniques. Compared with soil geochemistry and airborne radiometric methods, plant analysis is

more efficient in terms of ease of sampling, analytical cost, and can generate superior analytical data.

Dunn (1981) demonstrated through the analysis of twigs of black spruce from Saskatchewan that biogeochemical data outlined the buried U mineralization more closely than the soil geochemical data. The elevated U concentrations detected in spruce twigs were found to be related not to soil, but to bedrock U concentrations. Although biogeochemical methods have been applied with significant success to delineate ore deposits underneath Quaternary cover in Saskatchewan, British Columbia, and Nova Scotia (Dunn 1981, 1986 2007; Rogers and Dunn, 1993), this technique has yet to be applied in the till-covered regions of the Province of Newfoundland and Labrador, especially the boreal forests of Labrador that have semi-permanent permafrost. In addition to the thick till cover, the terrain in the Jacque's Lake area is rugged and covered by a dense coniferous forest, making it difficult to apply conventional geochemical sampling methods.

The second major aspect of this work is presented in chapters 3 and 4. Chapter 3 involves the evaluation of the U-accumulating potential of black spruce trees as a suitable biogeochemical sampling medium to locate buried ore in areas overlain by glacial till cover. Studies of U uptake and translocation to aboveground tissues through controlled greenhouse experiments are used to establish the biological accumulation coefficient and transfer factor for U in black spruce trees. The determination of a bioaccumulation coefficient for U in spruce trees also serves as an important metric for estimating radionuclide contamination in the terrestrial environment.

Metal bioaccumulation is defined as the net accumulation of a metal in a plant that results from exposure to a toxicant (EPA, 2004). Thus, bioaccumulation is a function of metal bioavailability, defined as the fraction of a metal species readily accessible to plant roots (NAS, 2000). Primarily, the transpiration stream, in addition to diffusive and microbial facilitated transport mechanisms designed to expedite nutrient uptake, control the uptake of ionic species across root membranes. The root membranes constitute the dominant route of metal uptake for plants in the terrestrial environment; however, foliar absorption of cations through leaf cuticle can be significant in areas impacted by the dry and wet deposition of airborne contaminants (Greger, 1999).

There is a lack of direct experimental data on U uptake rates and the bioaccumulation coefficients in spruce trees. The determination of the Biological Accumulation Coefficient (BAC), defined as the ratio of metal or ion concentrations in tissues or the whole plant to the available concentrations in the substrate under steady state conditions (Nergri and Hinchman, 2000; NAS, 2002), is a measure of a plant's capacity to accumulate the metallic ions. The tissue metal concentrations give an indication of the metal bioavailability in the underlying substrate. The value of BAC for a plant is controlled in part by a complex function of abiotic factors, including speciation and the solubility of metal cations, soil phyco-chemical properties (e.g. pH, redox potential, total organic matter content, etc.), and suspended particulate matter. Plant-specific properties also play significant roles in the uptake, translocation, and bioaccumulation of metallic elements.

Other studies have tended to measure metal bioaccumulation coefficients by analyzing field samples (i.e. soil, water, and plants) from aquatic and terrestrial environments (Robinson et al., 2006). Measurements of BAC using this approach have limited applicability due to variations in abiotic factors between different localities and environments. Additionally, the inability to adequately account for the quantities of metals taken up through the foliar uptake route severely negates an accurate estimation of the BAC in plants sampled from the natural environment. Consequently, the experimental approach in a controlled indoor setting and the use of identical substrate parameters produces more reliable BAC values. The magnitude of BAC determined in plants suggests: (i) the occurrence of the metallic species in forms readily available to biota, (ii) indications of the suitability of the plant for biogeochemical prospecting, and (iii) the possibility of applying in-situ botanical treatment to control metal contaminants.

The traditional approach for determining the BAC of metallic elements in plants relates the uptake of metal ions to the total metal concentration in soil rather than bioavailable concentration. The use of total metal concentration in substrates has several limitations. The most notable is that concentration ratios calculated as a function of total metal concentration in soil underestimate the biological accumulation coefficient and do not accurately reflect the uptake of mobile species by vegetation (Negri and Hinchman, 2000). In other words, this approach indirectly exaggerates the fraction of metal cations that is indeed bioavailable for plant uptake. To accurately evaluate concentration ratios for U in spruce trees, the uptake of U by black spruce seedlings grown on substrates treated with varied doses of uranyl nitrate hexahydrate in a controlled greenhouse

environment was investigated. The biological accumulation coefficient for U accumulation in spruce trees was calculated as a function of the available U concentration in the treated substrates.

Chapter 4 deals with the determination of the major U isotope ratios in spruce trees as tracers to fingerprint the true source and pathways of radionuclide contamination in environmental and geological samples. The main objective was to examine the ultimate source of metals absorbed by the spruce trees. That is, whether the metals taken up by vegetation were derived directly from the mineral phases in soil or till substrate, or were dissolved mineral constituents of groundwater taken up by plant root system. This part of the study involved the determination of the $^{238}U/^{235}U$ ratio in two-year-old black spruce seedlings grown in pots filled with 500 g substrate and treated with varied doses of depleted U.

Isotopic signatures of non-natural U (i.e., enriched or depleted in terms of ^{235}U) in environmental and geological samples are distinct from the $^{238}U/^{235}U$ ratio of natural U. Whereas, natural U has a fixed isotope ratio (=137.88) for its two major isotopes (^{238}U and ^{235}U), U derived from anthropogenic sources where the ^{235}U isotope was either enriched or depleted via isotope separation techniques has significantly different $^{238}U/^{235}U$ ratios. In particular, Depleted U (DU) has $^{238}U/^{235}U >> 137.88$. Analysis of the major isotope ratios is particularly important in establishing the actual source of U taken up by biogeochemical sampling media. Furthermore, the determination of the $^{238}U/^{235}U$ ratio in geological and environmental samples serves as an essential tool for discriminating contaminant sources and pathways. This technique also may provide

unique fingerprints of U contaminant type (i.e. weapons grade, depleted, fuel cycle) based on the extent of the isotope separation process and deviation from the natural ratio (i.e. 137.88) that are not obtainable from simple elemental solute concentration data (Roback et al., 2001). The determination of U isotope ratios in vegetation has important implications for biogeochemical prospecting for ore, as well as environmental monitoring and remediation.

References

Aksoy, A. and Demirezen, D. (2006). *Fraxinus excelsior* as a biomonitor of heavy metal pollution. Polish Journal of Environmental Studies, 15(1): 27-33.

Anand, R.R.; Cornelius M., and Phang C. (2007). Use of vegetation and soil in mineral exploration in areas of transported overburden, Yilgarn Craton, Western Australia: a contribution towards understanding metal transportation processes. Geochemistry: Exploration, Environment, Analysis, 7: 267-288.

Aspandia, M.F.; Anand, R.R., and Gray, D.J. (2008). Geochemical dispersion mechanisms through transported cover: implication for mineral exploration in Australia. Open File Report 246. CRM LEME, Bentley, WA 6102.

Batterson, M.J. and Liverman, D.G.E. (2001). The contrasting styles of glacial dispersal in Newfoundland and Labrador: Methods and case studies. Geological Society Special Publication No. 185. In McClenaghan M.B., Borowsky P.T., Hall G.E.M., Cook J.S. (Eds). Drift exploration in glaciated terrain.

Batterson, M.J., and Taylor, D.M. (2005). Till geochemistry of the Lititial Lake area, Central Labrador. Department of Natural Resources, Geological Survey, Open File 13L/0121.

Burgess, S.S.O.; Adam, M.A.; Turner, N.C. and Orig, C.K, (1998). The redistribution of soil water by tree root systems. Oecologia, 115: 306-311.

Butt, C.R.M.; Lintern, M.J., and Anand, R.R. (2000). Evolution of regolith and landscapes in deeply weathered terrain – implication for geochemical exploration. Ore Geology Reviews, 16: 167-183.

Caldwell, M.M.; Dawson, T.E. and Richards, J.H. (1998). Hydraulic lift: consequences of water efflux from roots of plants. Oecologia, 113: 151-161.

Cannon, H.L. 1961. Botanical prospecting for ore. Virginia Minerals 7(1): 1-11.

Cohen, D.R.; Kelly, D.L.; Anand, R.R., and Coker, W.B. (2010). Major advances in exploration geochemistry, 1998-2007. Geochemistry: Exploration, Environment, Analysis, 10: 3-16.

Cohen, D.R.; Silva-Santisteban, C.M.; Rutherford, N.F.; Garnett, D.L., and Waldron, H.M. (1999). Comparison of biogeochemical and stream sediment geochemical pattern in the northeastern region of NSW. Journal of Geochemical Exploration, 66: 469-489.

Cunningham-Dunlop, I.; Barbour, D.; Buschman, A.; Howson, S.; King, M.; Lennox-King. M.; Trevors, M. and Valenta, R. (2005). Assessment report on geological, geophysical, geochemical and diamond drilling investigation on the CMB Project,

Labrador, Canada. Aurora Energy Inc., Altius Resources Inc., and Frontier Development Group Inc.

Dell, B.; Bartle, J.R. and Tacy, W.H. (1983). Root occupation and root channels of Jarrah forest subsoil. Australian Journal of Botany, 31: 615-627.

Dion, M.; Loranger, S.; Kenneddy, G.; Courchesne, F., and Zeyed, J. (1993). Evaluation of black spruce (Picea mariana) as a bioindicator of aluminum contamination. Water, Air and Soil Pollution, 71: 29-41.

Dunn, C.E. (1981). The biogeochemical expression of deeply buried uranium mineralization in Saskatchewan, Canada. Journal of Geochemical Exploration, 15: 437-452.

Dunn, C.E. (1986). Biogeochemistry as an aid to exploration for gold, platinum and palladium in the northern forests of Saskatchewan, Canada. Journal of Geochemical Exploration, 25: 21-40.

Dunn, C.E. (2007a). Biogeochemistry in mineral exploration. Handbook of Exploration and Environmental Geochemistry, Volume 9. Elsevier.

Dunn, C.E. (2007b). New perspective on biogeochemical exploration. In Milkereit (2007) Ed. Proceedings of Exploration 07: Fifth Decennial International Conference on Mineral Exploration. Advances in Prospect-Scale Geochemical Methods, 249-261.

Dunn, C.E., Brooks, R.R., Edmondson, J., Leblanc, M. and Reeves, R.D. (1996). Bioeochemical studies of metal-tolerance plants from Southern Morocco. In: Anand R.R.; Cornelius M., and Phang C. (2007). Use of vegetation and soil in mineral exploration in areas of transported overburden, Yilgarn Craton, Western

Australia: a contribution towards understanding metal transportation processes. Geochemistry: Exploration, Environment, Analysis, 7: 267-288.

Govett, G.J.S. and Therden, P.R. (1987). Electrochemical patterns in surface soils – Detection of blind mineralization beneath exotic cover, Thalanga, Queensland, Australia. Journal of Geochemical Exploration, 28: 201-218.

Greger, M. (1999). Metal availability, uptake, transport, and accumulation in plants. In Prasad M.N.V (Ed). Heavy metal stress in plants from biomolecules to ecosystems. Springer-Verlag, Berlin. pp. 9-11.

Hamilton, S.M. (1998). Electrochemical mass-transport in overburden: a new model to account for the formation of selective leach geochemical anomalies in glacial terrain. Journal of Geochemical Exploration, 63, 155-172.

Hamilton, S.M. (2007). Major advances in soil geochemical exploration methods for areas of thick glacial drift cover. In Milkereit (2007) Ed. Proceedings of Exploration 07: Fifth Decennial International Conference on Mineral Exploration. Advances in Prospect-Scale Geochemical Methods, 263-280.

Hulme, K.A. and Hill, S.M. (2003). River red gums as a biogeochemical sampling medium in mineral exploration and environmental chemistry programs in the Curnamona Craton and adjacent regions in NSW and SA. Advances in Regolith 2003, 205-210.

Ives, J.D. (1978). The maximum extent of Laurentide Ice Sheet along the east cost of North America during the last glaciation. Arctic, 31: 24-53.

Kaste, J.M.; Heimsath, A.M., and Bostick, B.C. (2007). Short-term soil mixing quantified with fallout radionuclide. Geology, 35: 243-246.

Kerr, D.E. and Knight, R.D. (2007). Modeling overburden thickness in glaciated terrain: Lac de Gras, Northwest Territories, Canada. Milkerit B. (Ed). In Proceedings of Exploration 07: Fifth Decennial international Conference on Mineral exploration. Geophysical Inversion and Modeling. pp1073-1076.

Kim, J.N.; You, Y.J., and Chon, H.T. (2013). A biogeochemical orientation survey around a low-grade uranium deposit in black shale in the Goesan district, Korea. Geochemistry: Exploration, Environment, Analysis, doi: 10.1144/geochem2011-099.

Lintern, M.J.; Butt, C.R.M, and Scott, K.M. (1997). Gold in vegetation and soil – three studies from the goldfields of southern Western Australia. Journal of Geochemical Exploration, 58: 1-14.

Ma, Y. and Rate, A.W. (2009). Formation of trace element biogeochemical anomalies in surface soils: the role of biota. Geochemistry: Exploration, Environment, Analysis, 9: 353-367.

McClenaghan, M.B.; Thorleifson, L.H., and DiLabio, R.N.W. (1997). Till geochemical and indicator mineral methods in mineral exploration. In Gubin A.G. (1997). Proceedings of Exploration ’97. Forth Decennial International Conference on Mineral Exploration, 233-248.

National Academy of Sciences (2002). Bioavailability of contaminants in soil and sediments: Processes, tools and applications. The National Academies Press, Washington DC, pp 1-432.

Negri, C.M., and Hinchman, R.R. (2000). The use of plants for the treatment of radionuclides In: Raskin I., Ensley B.D. (Eds), Phytoremediation of toxic metals using plant to clean up the environment. John Wiley and Sons Inc., Toronto, pp118-123.

Pate, J.S.; Jeschke, D.W. and Aylward, M.J. (1995). Hydraulic architecture and xylem structure of the dimorphic root systems of south-west Australian species of *Proteacea*. Journal of Experimental Biology, 46: 907-915.

Poorter, H, and Nagile, O. (2000). The role of biomass allocation in the growth response of plants to different levels of light, CO_2, nutrients, and water: a quantitative review. Australian Journal of Plant Physiology, 27: 595-607.

Reid, N., and Hill, S.M. (2010). Biogeochemical sampling for mineral exploration in arid terrains: Tanami Gold Province, Australia. Journal of Geochemical Exploration, 104: 105-117.

Reid, N.; Hill, S.M. and Lewis, D.M. (2008). Spinifex biogeochemical expressions of buried gold mineralization: The great mineral exploration penetrator of transported regolith. Applied Geochemistry, 23: 76-84.

Reid, N.; Hill, S.M. and Lewis, D.M. (2009). Biogeochemical expression of buried gold mineralization in semi-arid northern Australia: penetration of transport cover at the Titania Gold Prospect, Tanami Desert, Australia. Geochemistry: Exploration, Environment, Analysis, 9: 267-273.

Robinson, B.; Bolan, N.; Mahimairaja, S., and Clothier, B. (2006). Solubility, mobility, and bioaccumulation of trace elements: abiotic process in the rhizosphere. In

Prasad M.N.V., Sajwan K.S., Naidu R. (Eds). Trace elements in the environment: biogeochemistry, biotechnology, and bioremediation. Taylor and Francis, London. pp 97-110.

Rogers, P.J., and Dunn, C.E. (1993). Trace element chemistry of vegetation applied to mineral exploration in eastern Nova Scotia, Canada. Journal of Geochemical Exploration, 48: 71-95.

Rogers, P.J., and Dunn, C.E. (1993). Trace element chemistry of vegetation applied to mineral exploration in eastern Nova Scotia, Canada. Journal of Geochemical Exploration, 48: 71-95.

Romheld, V.; Muller, C. and Marschner, H. (1984). Location and capacity of proton pumps in roots of intact sunflower plants. Plant Physiology, 76: 603-606.

Ryan, G.A. (1989). Native trees and shrubs of Newfoundland and Labrador. Parks Division, Department of Environment and Lands, Province of Newfoundland and Labrador. St John's.

Stirling, C.H.; Anderson, M.B.; Potter E-K. and Halliday, A.N. (2007). Low-temperature isotopic fractionation of uranium. Earth and Planetary Science Letters, 264: 208-225.

Stone, E.L. and Kalisz. P.J. (1991). On the maximum extent of tree roots. Forestry Ecology Management, 46: 59-102.

Sylvia, D.M.; Hartel, P.G.; Fuhrmann, J.J. and Zuberer, D.A. (2005). Principles and applications of soil microbiology, (2nd ed). Pearson Prentice Hall, New Jersey.

Weyer, S.; Anbar, A.D.; Gerdes A.; Gordon, G.W.; Algeo, T.J., and Boyle, E.A. (2008). Natural fractionation of $^{238}U/^{235}U$. Geochimica et Cosmochimica Act, 72:345-359.

Chapter 2

In

Praise K. Nyade, Derek H.C. Wilton, Henry P. Longerich, Gary M. Thompson, and Paul McNeill (2013). Use of surficial geochemical methods to locate areas of buried uranium mineralizations in the Jacque's Lake area of the Central Mineral Belt, Labrador, Canada. Canadian Journal of Earth Sciences, 50: 1-13 [Published online] dx.doi.org/10.1139/cjes-2012-032.

Use of surficial geochemical methods to locate areas of buried uranium mineralization in the Jacque's Lake area of the Central Mineral Belt, Labrador, Canada

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Abstract

Surficial geochemical methods were applied to delineate zones of anomalous uranium and related element concentrations in areas overlain by an extensive blanket of glacial sediments and dense vegetation cover in the Jacque's Lake area of the Central Mineral Belt, Labrador, Canada. The study involved sampling and analyses of vegetation including: black spruce twigs and bark, and Labrador tea shoots. Ash derived from the vegetation samples was analyzed using ICP-MS following ignition at 450 ^{o}C for a suite of 46 elements. B-horizon soil was analyzed following aqua regia digestion to investigate the surface expression of the bedrock mineralization. Uranium and pathfinder element (e.g. Pb, V, Sr, and Mo) concentrations were low in the <250 μm fraction of B-horizon soils. Area with anomalous U concentration in the <250 μm sized-fraction of B-horizon

soils coincide thin overburden material (i.e. <15 cm) and U concentration varied from 50 ppm to 405 ppm. Biogeochemical signatures of the bedrock mineralization in black spruce twigs produced greater anomaly to background contrasts for U and pathfinder elements (e.g. Be, Ag, Pb, Ca, and Sb) and correlated more precisely with the detected radiometric U/Th anomaly than the soil responses. Principal component analysis of spruce twig data discriminated three major components, including, plant nutrients, ore-related elements, and a mobile species component. Uranium concentration varied from less than the detection limit (0.01 ppm) to 23 ppm U in black spruce bark and ranged from less than the detection limit in approximately 40% of samples to 18 ppm in Labrador tea stems.

2-1 INTRODUCTION

Mineral exploration in the northern hemisphere, in particular, Labrador is hampered by the low abundance of bedrock outcrops and a variable thickness of sediments associated with the various episodes of glaciation. The depths and compositions of the glacial deposits present special problems for surficial exploration geochemistry. This is because the overburden materials in most glaciated regions may be distinct from the bedrock geochemistry and thus host geochemical signatures that are unrelated to the underlying bedrock (Dunn, 1981). Consequently, the exogenous material may mask the surface expression of the underlying bedrock features (Dunn, 1981, 2007a; Brooks et al., 1995; Anand et al., 2007; Reid and Hill, 2010).

Deep penetrating methods designed to detect subtle geochemical signals produced by dispersion of labile mineral components from buried ore have the potential to observe obscured geological features. Many such techniques are not only costly but have an environmental impact (Dunn, 2007a). Biogeochemical methods using plants as sampling media for mineral exploration provide a surface expression of buried geological features. Analysis of vegetation has been effectively demonstrated to detect buried mineralization globally (e.g. Cannon, 1961; Dunn, 1981, 2007a; Cole, 1991). Plants have the unique advantage of taking up mineral constituents via absorption, adsorption, and cation exchange processes from weathered bedrock and associated groundwater using deep penetrating root systems (Cannon, 1961; Brooks, 1983; Lintern et al., 1997; Arne et al., 1999; Hulme and Hill, 2003; Anand et al., 2007; Reid et al., 2008) and, therefore, act as a composite integrating sampling medium for buried geological features. Studies in South

Africa on root penetration and water extraction from a deeply weathered granite profile by *Eucalyptus grandis* found that young trees can extract water from depths of up to 8 m, whereas older tree roots can extract from depths greater than 8 m (Dye, 1996 *in* Aspandia et al., 2008).

This study presents the results from an orientation survey over an area with elevated U/Th anomalies (Cunningham-Dunlop et al., 2005) in the Jacque's Lake area of the Central Mineral Belt, Labrador. The objective was to apply a combination of surface geochemical methods to locate buried mineral targets in the glacial till-covered terrain by (i) sampling and analyzing surface media, including soil, and vegetation, and (ii) evaluating and comparing surface geochemical signatures with data generated in an airborne radiometric survey of the Jacque's Lake area. Black spruce (*Picea mariana*) and Labrador tea (*Rhododentron tomentosum*) were selected for this study due not only to their abundance and wide distribution but more importantly to their significant metal accumulating properties (Dunn, 2007a; Brooks, 1972). Soil was analyzed for U and other elements, in order that direct comparison with vegetation biogeochemistry could be made.

2-2 PHYSICAL SETTINGS

The Jacque's Lake prospect is located in the Central Mineral Belt (CMB), near the east coast of Labrador, Canada (Fig. 2-1). Uranium mineralization was first reported from the Makkovik area of the CMB in 1954 (Gandhi, 1978), and subsequent exploration led to the discovery of a number of significant occurrences, notably the Kitts, Michelin, Inda, and Burnt Brook deposits. The Jacque's Lake prospect, with an average grade of 0.32 %

U_3O_8, was discovered in 1956 by the British Newfoundland Exploration Limited, (Brinex) (Gandhi, 1978; Kerr and Sparkes, 2009). The area is located 30 km southwest of the coastal village of Postville and can only be accessed by helicopter, floatplane, or snowmobiles in winter. The climate is semi-arctic, with typical daytime temperatures in winter ranging between -10 and -30 ^{o}C; annual snowfall is up to 400 mm. The summer season is usually short, cool, and wet, with daily temperatures ranging between10 and 25 ^{o}C.

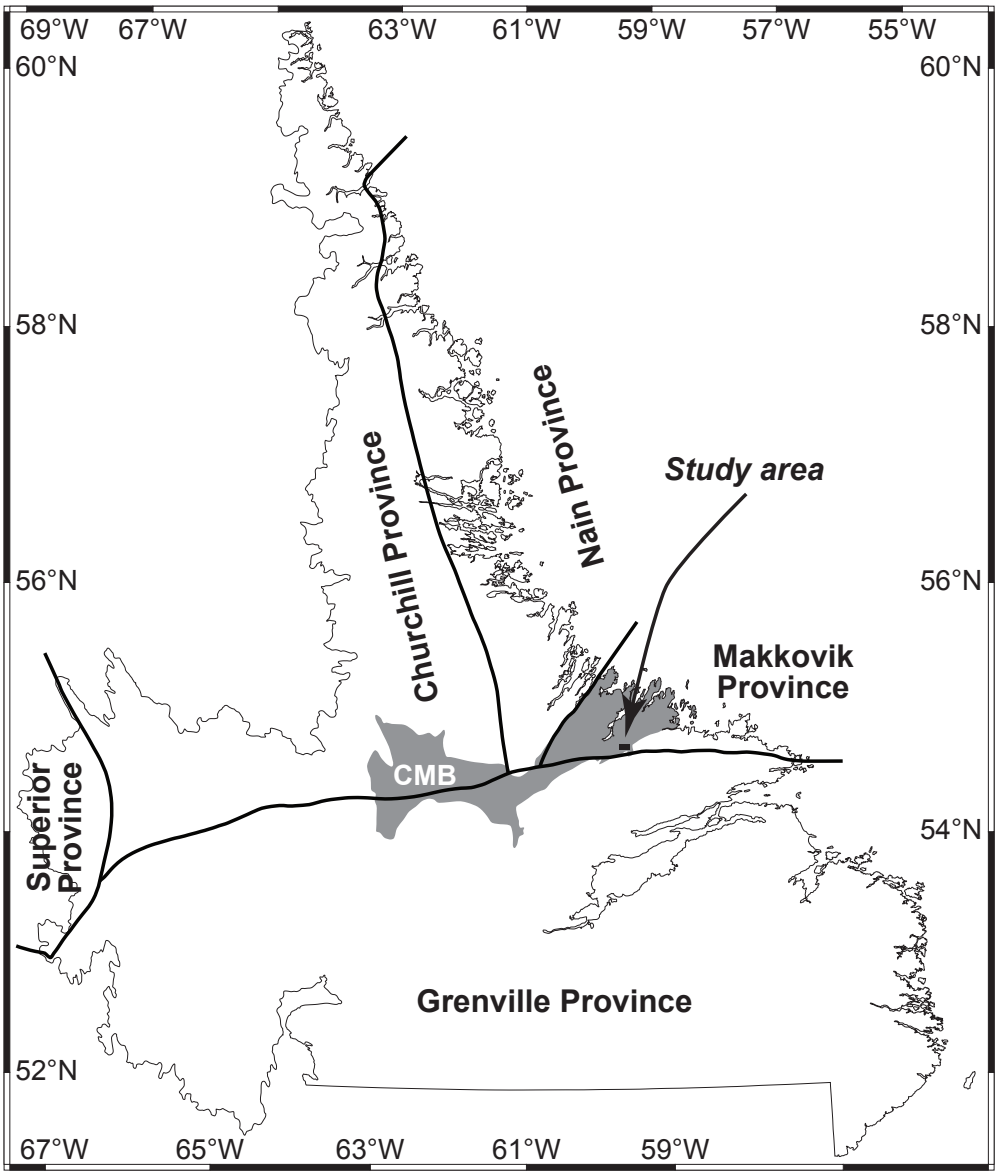


Figure 2-1: Location map of the Jacque's Lake study area in the Central Mineral Belt (CMB), Labrador.

Ponds, small lakes, and bogs are widespread in the low-lying areas. Bedrock outcrops are sparse and limited to the southern part of the study area. Based on the Canadian System of Soil Classification (Agriculture Canada, 1998), soils of this area are generally described as podzols (orthic ferro-humid).

Vegetation in the Central Mineral Belt is typical of the boreal ecoregion with very low flora diversity, consisting of a mosaic of successional and subclimax plant communities (Ryan, 1989). The Jacque's Lake study area is densely vegetated by a coniferous forest cover in which black spruce (*Picea mariana*) is the dominant species. Other common but sparsely distributed tree species, include white spruce (*Picea glauca*), balsam fir (*Abies balsamea*), and tamarack (*Larix laricina*). The understory is covered principally by shrubs, namely, Labrador tea (*Rhododendron tomentosum* and *Rhododendron groenlandicum*) and mountain alder (*Alnus rugosa* and *Alnus crispa*); and bryophytes and ferns in bogs.

The surface P-horizon is composed of peat, a partially decomposed woody and mossy vegetative matter that can accumulate up to 1 m thick in bogs and wetlands (Cunningham-Dunlop et al., 2005). A brown to dark coloured organic-rich A-horizon lies below the P-horizon that varies in thickness from 5 to 12 cm, except in low-lying areas where it is up to 25 cm thick. The A-horizon is underlain by a grey to brownish coloured B-horizon diamicton composed of loosely packed stony and amorphous to fine-grained sandy material. The C-horizon is not well developed in the study area. The soil is generally well drained at higher elevations.

2-2-1 GLACIAL HISTORY AND QUATERNARY GEOLOGY

As in most parts of Canada, the Laurentide Ice Sheet covered the study area during the last period of glaciation. The pattern of glaciation and the glacial dispersal of materials were interpreted in regional surveys by Ives (1978), Liverman et al. (1993), Batterson and Taylor (2004, 2005) and McCuaig and Smith (2005). Ice flowed northeast from Central Labrador to the coast through the major valleys that cut through mountain ranges (Batterson and Taylor, 2004). Till is the dominant glacial deposit in the Central Mineral Belt. The poorly sorted glacial deposit is composed of fine silt to coarse sand. Clasts range in size from pebbles of 0.5 cm in diameter to boulders of up to 200 cm in diameter and are mainly sub angular to angular (McCuaig and Smith, 2005). Valleys are filled with thick sequences of glaciofluvial outwash and boulders deposited during the glacial retreat. The surface tills and glaciofluvial outwash are commonly underlain by sediments, deposited during periods of high sea level following deglaciation (Batterson and Taylor, 2004; McCuaig and Smith, 2005).

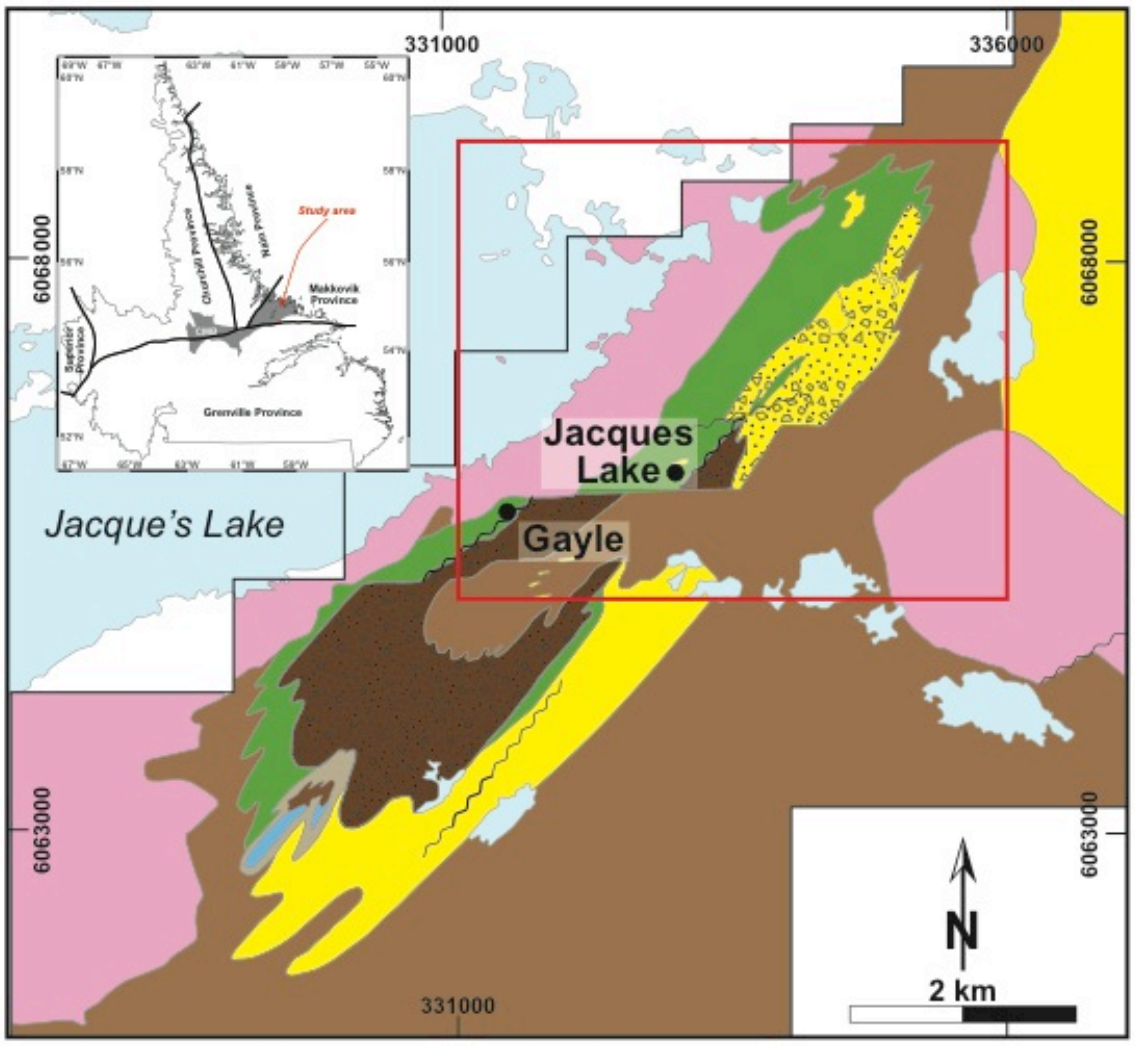
2-2-2 BEDROCK GEOLOGY

The Central Mineral Belt of Labrador consists of six successive Proterozoic supracrustal sequences, Archean basement rocks, and associated granitoids that occupy a 260 km x 75 km region in central to coastal Labrador; the region is essentially the northeastern margin of the Laurentian Shield (Wilton, 1996). Uranium mineralization in the Jacque's Lake study area is hosted by *ca.* 1856-1883 Ma rocks of the Aillik Group (Hinchey and LaFlamme, 2009). Regionally, the Aillik Group "comprises interbedded

sandstone and siltstone, conglomerate, tuffaceous sandstone, felsic tuff, rhyolite, volcanic beccia, and lesser mafic volcanic rocks and volcaniclastic sedimentary rocks" (Hinchey and LaFlamme, 2009) intruded by synvolcanic granitoids.

Detailed mapping of the Aillik Group in the Jacque's Lake area was conducted by Aurora Energy Corporation geologists following definition of the radiometric in the 2007 survey (Cunningham-Dunlop and Lee, 2008). The general stratigraphy of the occurrence as outlined by the mapping is illustrated on Fig. 2-2. The dominant rock type underlying the immediate vicinity of the mineralization is fine-grained, dark greywacke. However, the mineralization is hosted by magnetite-bearing intermediate volcanic rocks (trachyandesite), which are variably folded (Cunningham-Dunlop and Lee, 2008). The footwall rocks to the mineralized zone are post-tectonic granitoids, and the conglomerate with clasts of the trachyandesite comprises hanging wall strata (i.e., the stratigraphy is not overturned). Deformed mafic intrusive rocks cut the mineralized horizon.

The uranium mineralization comprises fracture fillings cutting the host intermediate volcanic rocks. The mineralized zone has been defined over a length of 220 m. Cunningham-Dunlop and Lee (2008) suggested that the mineralization is epigenetic.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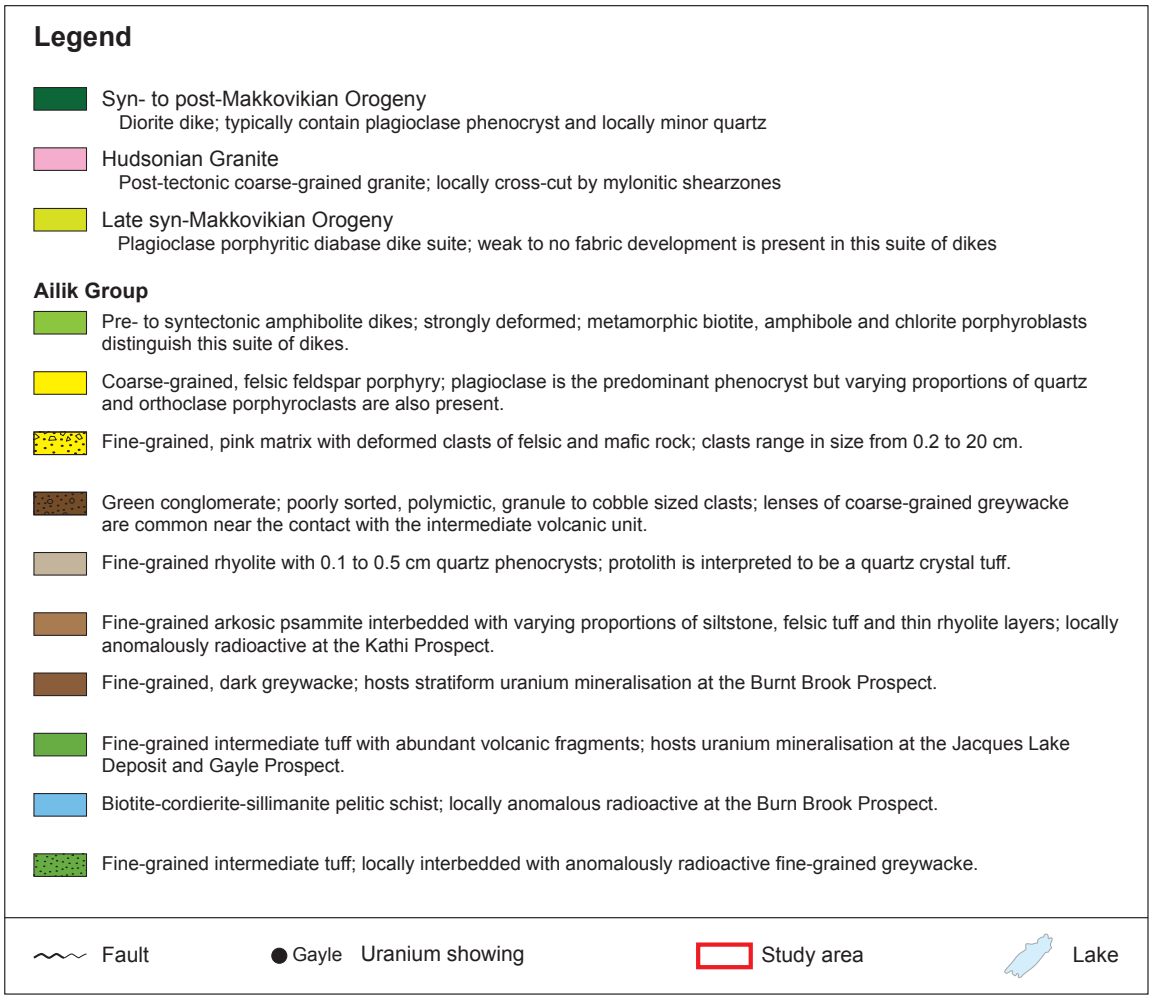

Figure 2-2: Geology of the Jacques Lake area of the Central Mineral Belt, Labrador with locations of uranium prospects. Legend (modified after Bailey, 1977) [UTM NAD 83 Zone 21; MapInfo Professional, version 10.0].

2-3 MATERIALS AND METHODS

2-3-1 REAGENTS AND MATERIALS

The nitric acid (70 % *v/v*), hydrochloric acid (38 % *v/v*), and hydrogen peroxide used for dissolution of plant ash were of analytical grade and supplied by Fisher Scientific (Fair Lawn, New Jersey, USA). The acids were distilled prior to use and diluted with deionized water (Nano-pure water, 18 mΩ cm @ 20 ^{o}C) obtained from a Millipore Elix-5/Mill-Q water purification system (Bedford, Ontario, Canada). Single element standard solutions obtained from SCP Science, St-Laurent, Quebec, Canada, were used for calibrations and quality control. Standard reference materials included SRM-2710a (Montana I soil), SRM-2711a (Montana II soil), SRM-1547 (peach leaves), and SRM-1575 (pine needles) from the National Institute of Standards and Technology (NIST, (Gaithersburg, USA). Vegetative radionuclide reference materials, namely, CLV-1 (spruce twigs) and CLV-2 (spruce needles) were obtained from the Canadian Certified Reference material Project, CANMET Mining and Mineral Sciences Laboratories, Ontario,Canada.

2-3-2 EQUIPMENT AND APPARATUS

Plant samples were dried in Fisher Scientific Thermo scientific (Menetta, Ohio, USA) gravity convection ovens (Model 664) and pulverized using a tungsten carbide cup mill (TE 100/250, Angsrom Inc., Chicago, Illinois, USA). The inductively coupled

plasma optical emission spectroscopy (ICP-OES) was a PerkinElmer Inc. (Norwalk, Connecticut, USA) Optima 4300. The inductively coupled plasma mass spectroscopy (ICP-MS) instrument was a PerkinElmer Sciex (Ontario, Canada) ELAN DRC II equipped with nickel sampling and skimmer cones and a Seaspray U-series nebulizer fitted into a standard cyclonic spray chamber. Plant samples were ignited in a programmable muffle furnace (PSH Kilns and Furnaces, Oakville, Ontario, Canada).

2-3-3 AIRBORNE RADIOMETRIC SURVEY

Fugro Airborne Survey Corporation undertook an airborne radiometric survey over the Jacque's Lake property on behalf of Aurora Energy Limited, St John's, Newfoundland in 2007. The detected U/Th radiometric responses constitute the baseline for the present study.

2-3-4 SAMPLE COLLECTION

Vegetation samples were collected in August of 2009 on a northeast-oriented grid centered over the detected U/Th radiometric anomalies at the Jacque's Lake area. The grid consisted of 10 lines spaced at 500 m intervals with each line extending approximately 1600 m and oriented NW. Black spruce twigs (composed of stems and needles) 8 to 12 cm in length, corresponding to approximately 7 - 10 years of new growth at the top of the tree (Dunn, 2007a), were sampled with Teflon-coated pruning shears. Sample sites were spaced at approximately 100 m intervals and sampling was performed from a helicopter. Sample coordinates were recorded with a handheld global positioning

system (GPS) tracking device (Rhino 120, Garmin, Olathe, Kansas, USA) in NAD North American Datum 83 Zone 21. A total area of 8 km^2 was covered at a sampling rate of 24 samples per hour. Ground level vegetation samples, comprising Labrador tea stems with leaves and black spruce bark, were collected at intervals of 100 m along two northwest-oriented traverses aligned perpendicular to the detected radiometric anomaly. A total of 262 plant samples were collected and each stored in pre-labelled plastic bags.

The B-horizon soils were sampled by Aurora Energy Ltd. across the major radiometric anomaly that defined the southern part of the property in the summer of 2005. The B-horizon soils were collected to allow for consistency in the samples, since the C-horizon is not well developed in some parts of the study area. Soils were obtained from depths of 15-25 cm along fifteen 1400 m long northwest oriented lines spaced 50 m apart. Approximately 30 samples were collected along each line. Silt samples were collected in areas where sample stations intersected streambeds. A total of 322 B-horizon soils, and 34 silt samples were collected. Samples were stored in labelled plastic bags.

2-3-5 SAMPLE TREATMENTS

The soil and silt were air-dried to constant weight and were sieved to separate the <250 μm size fraction for analyses. Both sets of samples were submitted for multi-element analysis using ICP-MS and ICP-OES at Actlabs, Ancaster, Ontario, Canada. Plant samples were oven dried at 75 ^oC for 2 weeks prior to air-drying and were pulverized using a tungsten carbide cap mill (TE 100/250, Angsrom Inc., Chicago). The cap and ball of the grinder were cleaned with compressed air and ethanol between

samples to minimize cross contamination from previous samples. The resultant powders were placed in labelled clean airtight plastic containers and stored in desiccators.

2-3-6 ANALYSIS OF SAMPLES

The <250 μm size fractions of B-horizon soil and silt samples were analyzed at Actlabs, using ICP-MS and ICP-OES (Perkin OPTIMA 4300) following aqua regia digestion (0.5 mL H_2O, 0.6 ml concentrated HNO_3, and 1.8 mL HCl) of approximately 0.1 g samples. The ICP-MS instrument parameters were as follows: radio frequency (RF) power (1250 W), nebulizer gas flow (1.05 L/min), auxiliary gas flow (1.0 L/min), and plasma gas flow (15 L/min). The mass spectrometer was quadrupole-based with dual pulse counting and analog detector modes. Calibration solutions and reagent blanks were run after every 13 samples.

Approximately 1.0 g of each plant powder was ignited at 450 ^{o}C following which the ash was dissolved in 2 mL of 8 mol/L HNO_3. The resultant solution was gravimetrically diluted to 60g for analysis for a suite of 46 elements on an ICP-MS (ELAN DRC II, PerkinElmer Sciex, Ontario, Canada). A set of four certified reference materials, including (i) SRM-1547 (peach leaves), SRM-1575 (pine needles), (ii) CLV-1 (spruce twigs), and (iii) CLV-2 (spruce needles) CCRMP, CANMET Mining and Mineral Sciences Laboratories, Canada, and reagent blanks were included in each batch of analyses. Element enrichments in plant parts are presented as median and maximum concentrations in Table 2-1. Despite the low detection limits available for ICP-MS analyses, a number of elements, including Cl, Se, Br, I, Hg, As, and Bi, were below their respective detection limits.

Table 2- 1: Median and maximum concentrations of elements determined in ash of plants parts sampled from the Jacque's Lake areas o f CMB, Labrador.

Analyte	Spruce twigs		Spruce bark		Spruce needles		Labrador tea stems		Labrador tea leaves	
	Median	Maximum	Median	Maximum	Median	Maximum	Median	Maximum	Median	Maximum
Li	0.57	47.2	0.08	0.5	0.004	0.8	0.2	0.7	0.04	0.08
Be	0.03	0.54	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002
B	13.7	225	9.2	12.6	6.9	31	11.5	24.2	5.8	6.7
Mg (%)	0.11	2.53	0.054	0.088	0.02	0.077	0.059	0.012	3	0.04
Al	63.3	931.5	51.1	150	14	40.6	44.6	80	18.7	36
Si	5.3	31	1.3	11.2	<0.004	<0.004	10.5	44.7	<0.004	<0.004
P (%)	0.1	2.4	0.03	0.1	0.06	0.09	0.06	0.1	0.04	0.06
S (%)	0.05	1.3	0.002	0.04	0.001	0.1	0.02	0.07	ND	ND
Ca (%)	0.035	7.2	1	1.6	0.11	1.8	0.2	0.4	0.14	0.18
Ti	4.7	78	1.2	1.8	0.06	9.3	4.2	6.3	1.5	4
V	0.07	0.8	0.02	0.7	0.009	0.4	<0.001	<0.001	<0.001	<0.001
Cr	0.9	56.9	1.5	56.9	0.6	22	0.3	0.4	0.5	1.1
Mn	401	6686	606	1364	154.9	761.5	503	644	376	643
Fe	35.6	1191.2	15.7	36	12.3	62	134	735	20.3	34.7
Co	2.3	120.5	0.9	6.3	0.3	41	0.5	0.9	1.4	7.4
Ni	3.1	54.4	0.9	2	1.3	18	1.8	8.8	1.9	27.3
Cu	8.2	102	1.5	4.3	5.1	28	7.9	14.5	2.9	3.6
Zn	47.5	790.6	79.4	156	19.2	177	14	34	14.7	24.6
Rb	27	488.3	4.7	13.8	7.4	306	13.4	25.1	10.7	21.8
Sr	12.4	362.6	24	50.6	0.2	39	3.9	19.2	5.6	21.8
Mo	0.2	7	0.1	0.3	0.4	1.6	0.035	0.05	0.1	0.4
Ag	0.12	19.7	0.2	0.05	0.026	3.8	<0.0001	<0.0001	<0.0001	<0.0001
Cd	0.3	44	0.07	0.5	0.09	0.6	0.004	0.021	0.005	0.03
Sn	0.2	0.4	0.4	0.7	0.15	0.7	0.06	0.073	0.26	0.4
Sb	0.01	0.23	0.003	0.03	<0.0001	<0.0001	<0.0001	<0.0001	0.02	0.09
Cs	0.4	10.8	0.08	0.6	0.06	2.1	0.037	0.12	0.16	0.7
Ba	21.3	263.2	122	258.5	8.3	102	71.5	116.8	58.7	88.5
La	0.02	0.5	0.006	0.03	0.07	0.9	0.04	0.11	0.013	0.09
Ce	0.05	1	0.01	0.026	0.03	0.44	0.09	0.23	0.021	0.12
Tl	0.8	3.9	0.03	0.07	0.1	1.9	<0.001	<0.001	0.017	0.09
Pb	0.14	2.8	0.17	0.38	0.2	1	0.18	0.34	0.08	0.16
U	17	61	3.1	23	0.1	1.3	1.4	17.8	0.04	0.85

All values are concentrations in ppm unless otherwise indicated.

2-4 RESULTS

The airborne radiometric survey provided high-resolution data for discriminating uranium mineralization in the study area. Spatial plots of the radiometric data highlight a prominent U anomaly in the southeastern part of the property and other smaller magnitude U anomalies to the north (Fig. 2-3). The elevated radiometric anomalies detected in the southern part of the property are proximal to northeasterly-trending fault structures. These anomalies extend to a slope break adjacent to a prominent ridge with approximately 150 m relief. The overburden at this southern limit is relatively thin, ≤2 m, and extends eastwards to zones covered by sphagnum moss bog.

The central part of the property contains an elongate northwest-trending U/Th anomaly that is 500 m wide and 3000 m long. Elevated U/Th anomalies cover an area that is approximately 750 x 300 m located at the southwestern part of the property where there is <2 m of till cover. Zones of peak radiometric signals recorded U/Th activities ranging between 818 to 1150 cps^{-1} (counts per second) with widths of up to 5 m (Fig. 2-3). Areas overlain by thick glacial sediments and dense vegetation cover recorded U/Th activities < 120 cps^{-1}.

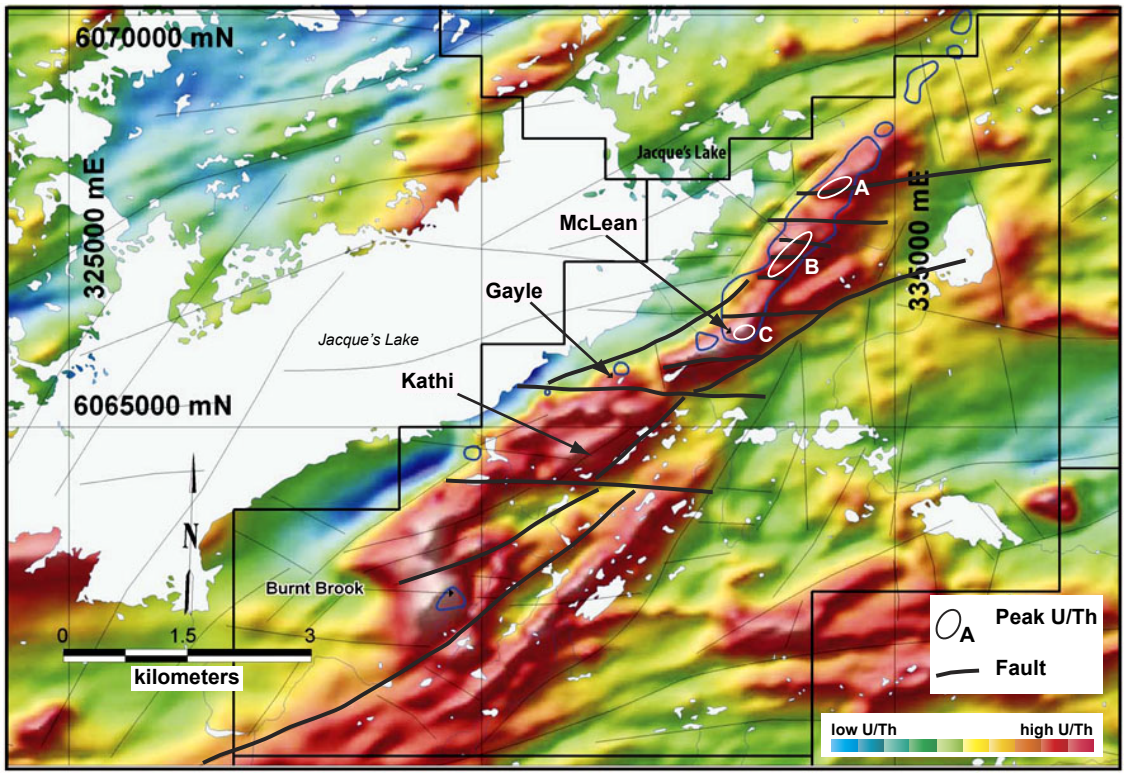

Figure 2-3: Spatial plot of airborne radiometric responses of U mineralization at the Jacque's Lake area and adjoining U showing at Gayle, Kathi, McLean, and Jacque's Lake. Points A, B and C represent areas with peak U/Th anomalies. [UTM NAD 83 Zone 21, Geosoft Oasis Montaj, version 6.4.2].

2-4-1 VEGETATION BIOGEOCHEMISTRY

The data for ignited standard reference samples (SRM 1575, SRM 1547), and a few selected black spruce twig samples exhibit losses through volatilization of up to 80% for some elements, including As, Se, and Hg, and minor losses (<10%) for Zn when compared with data generated from acid digested samples (Appendices 2-1 and 2-2).

Volatilization of U and Pb during dry ashing was insignificant based on 94% to 97% recoveries of the elements from certified reference materials, CLV-1 and CLV-2 (Appendix 2-3). Bromine and I concentrations were below their individual analytical detection limits in all ignited reference samples, suggesting high losses through volatilization.

Dunn et al. (1995) undertook the first and only known reconnaissance biogeochemical survey in the Province of Newfoundland and Labrador using the ash of twigs of black spruce trees sampled from the Baie Verte region. There is no other known documented account utilizing plant biogeochemistry for mineral exploration in Labrador. In the current study, ash yield after ignition of the various plant parts is presented in Table 2-2. Mean accumulation of U in various plant parts exhibited the following variations: black spruce twigs > black spruce bark > stem bark > Labrador tea stem > spruce needles > Labrador tea leaves. Compared with spruce twigs and bark samples, U enrichment in Labrador tea stems and leaves is substantially low. For example, approximately 76 % of Labrador tea samples (stem and leaves) exhibit U concentrations near the background concentration of 4.7 ppm and also display little anomaly to background contrasts in areas with elevated radiometric U/Th anomalies. The highest U concentration of 9 ppm in Labrador tea leaves is from a plant collected approximately 500 m southwest of the elongate radiometric U/Th anomaly on line L6. In addition, concentrations of elements, including Zn, Cu, Pb, Co, Ni, Ag, Fe, and Mo, in Labrador tea stems and leaves are below their estimated global mean concentrations in plants (Dunn, 2007b).

Table 2- 2: Percent ash recovery after ignition of approximately 1 g plant samples at 450 ^{o}C

Sample	Ash Yield (%)	Mean (%)
Labrador tea stem	5.8-11.7	8.8
Labrador tea leaves	6.2-12.3	9.2
Spruce bark	8.9-16.0	12.1
Spruce twigs	8.2-13.6	10.4
Spruce needles	9.4-15.8	11.6

The extent of metallic element enrichments in spruce bark, twigs, and needles varied markedly. For instance, concentrations of Al, Mg, Rb, Zn, Mn, and Cd are statistically significantly ($P < 0.01$) higher in bark samples than their respective concentrations in twig and needles. In contrast, Be, Ca, Sb, U, and V are considerably enriched in twigs and linearly correlated. Uranium concentrations in the bark samples exhibit good to moderate associations with Pb, Ag, and Mo defining correlation coefficients ranging between 0.5 and 0.76. While uranium contents in bark and twigs are above the analytical detection limit in more than 90% of the samples, the metal content in needles is observed to be predominantly below the analytical detection limit (0.02 ppm) except in a few samples where the metal concentrations ranged between 0.1 and 1.3 ppm.

Spruce twig data exhibit a good correlation between U and Ag, Be, Pb, Sb, Ce, Tl, Ca, and V. Areas with elevated U concentrations in spruce twigs correlate with the northeast-southwest U/Th radiometric anomaly that defines the Jacque's Lake prospect (Fig. 2-4A). Median and maximum U concentrations in black spruce twig samples are 17 and 61 ppm, respectively, with a threshold concentration (95 percentile) set at 28 ppm. A

student's t-test (95% CI (confidence interval)) of means and threshold concentrations of U spruce twigs and bark samples showed significant differences ($P < 0.01$) suggesting preferential accumulation in twigs compared with bark.

The most pronounced uranium accumulations in spruce twigs are in samples collected in the central and southern parts of the study area and to the north by the circular peak anomaly (Fig. 2-4B). Uranium concentrations from the southern subarea, which is characterized by thin glacial overburden, ranged between 11 and 37 ppm. The prominent peak U anomaly in the southeastern zone (Fig. 2-4B) is defined by U concentrations ranging between 16 and 23 ppm. Spruce twigs sampled from the central portion of the study area, specifically, along traverses L5–L6 are the most enriched in U and exhibit good correlation with the radiometric U/Th anomalies. Concentrations of the metal in samples from this area ranged from 26 to 61 ppm and are significantly associated with Ag, Pb, and Ca. Piercey (2009) also reported strong associations of Ag with Pb and U in drill core lithogeochemical data of the Jacque's Lake prospect.

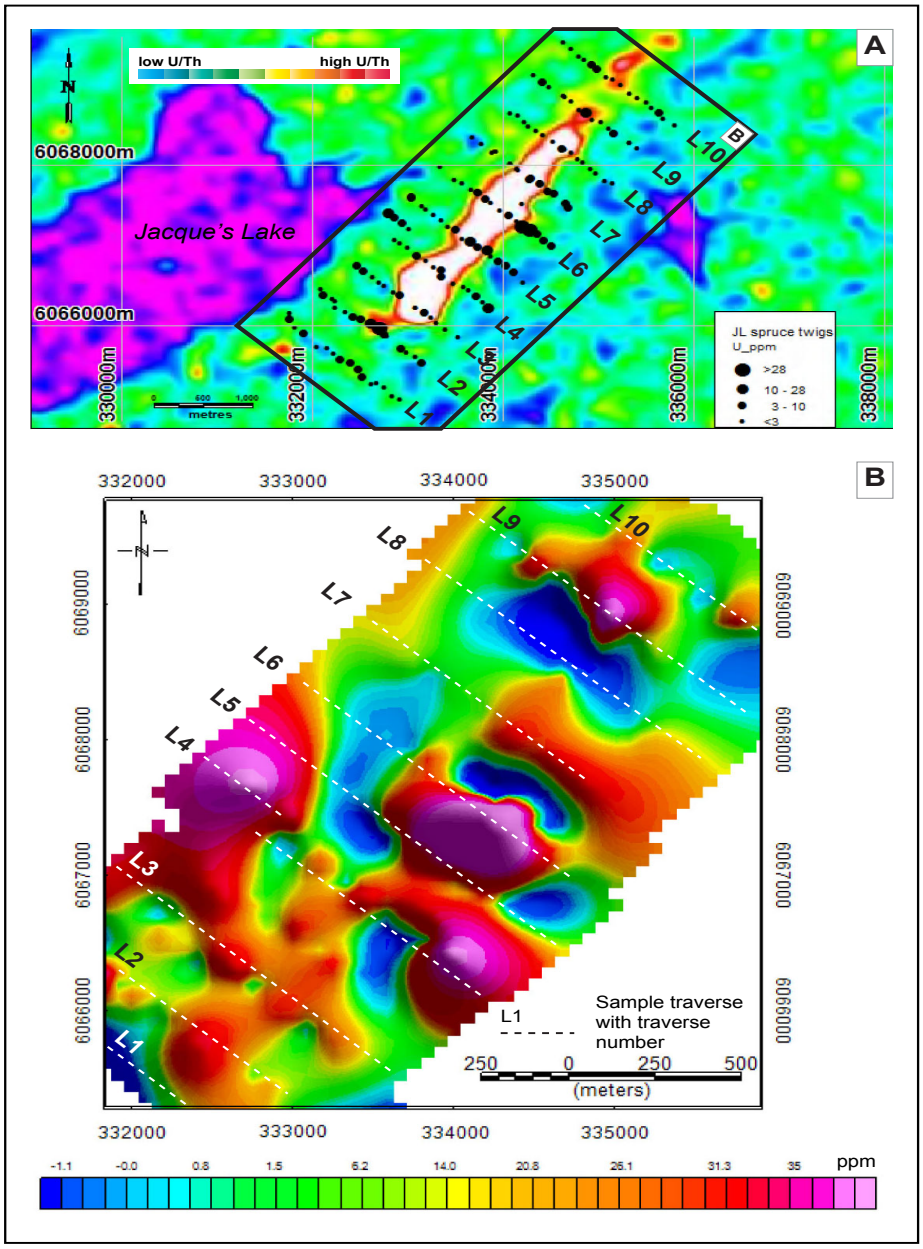

Figure 2-4: (A) Spatial plot of uranium concentration in spruce twigs compared to the airborne radiometric U anomaly; peak U is denoted by red color. (B) Spatial plot with possible dispersion patterns of uranium concentration in spruce twigs sampled from the Jacque's Lake area of the Central Mineral Belt, Labrador. [UTM NAD 83 Zone 21; biogeochemical data were gridded using Geosoft Oasis Montaj version 6.4.2].

Anomaly-to-background contrasts of Ag are high in twigs with concentrations ranging between 16 ppm and 142 ppm. Lead concentrations in twigs range between 0.06 and 114 ppm with a median of 19 ppm, and a threshold concentration (95 percentile) of 38 ppm. Associations of V, Ce, Pb, and Ca with U are not uncommon since these elements constitute important components of U minerals, e.g. carnotite, davidite, curite, and uranophane. Dunn (1981) reported associations of Be and Pb with U in spruce twigs collected from areas in Saskatchewan, Canada. Elements, including Fe, Pb, C, Mo, Zn, Co, and Mn are highly enriched in twigs sampled from the vicinity of the major northeast-oriented faults (Fig. 2-2). In contrast, Ti, K, Ba, and Si are depleted in twigs collected directly over and in the immediate vicinity of the faults.

Principal component analysis of spruce twig biogeochemical data discriminated three major components that account for approximately 72% of the total variability (Table 3). The first factor accounting for 5% of the total variance has high positive loadings for Zn, Ca, P, B, Mn, Mo, Cr, Fe, Co, Rb, and Sr. The second factor constrained Sn, Pb, and Cu in an inverse relationship with Si and S (Fig. 2-5); whereas Ag, U, Sb, Ca, V, and Cd constitute the third factor. Figure 6 shows the component plot of factors 1 and 2 in space.

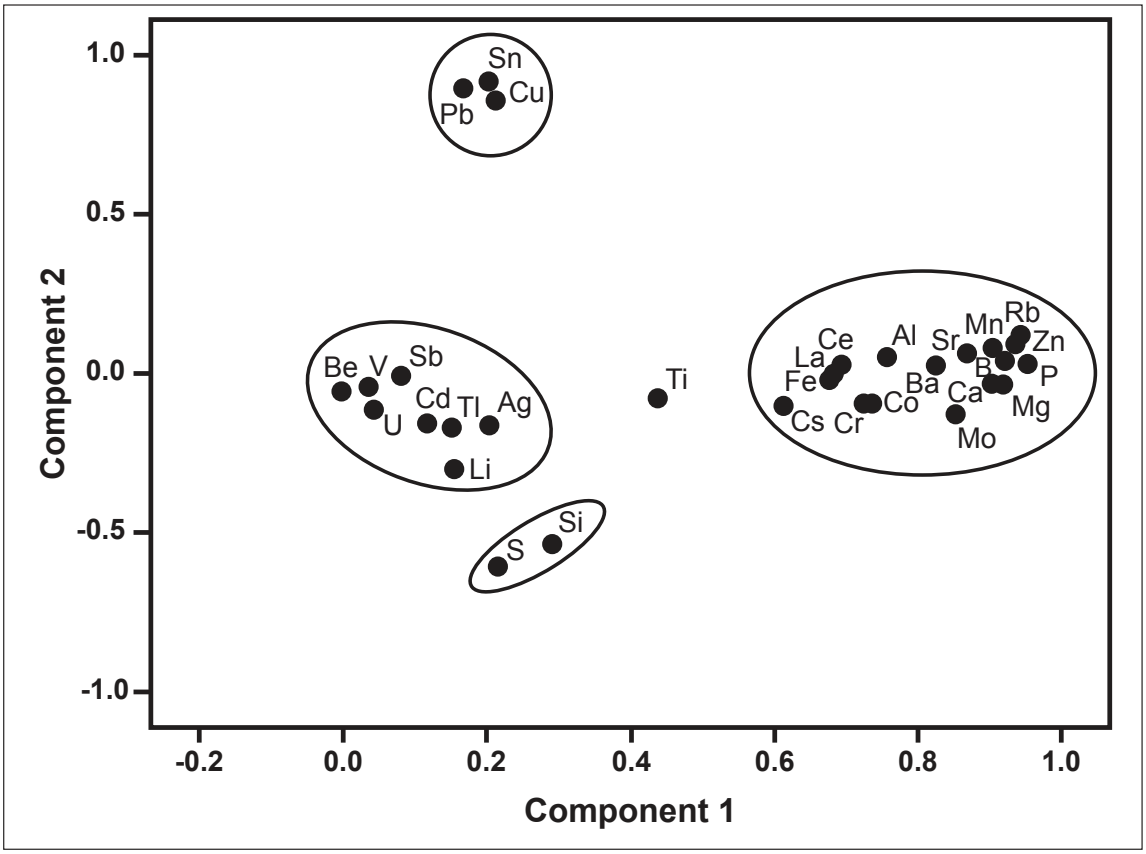

Figure 2-5: Principal component plot of geochemical associations between metallic element concentrations in black spruce twigs.

Table 2- 3: Principal components of elements associations in black spruce twig biogeochemical data

Components	% Variance	Elements
1	56.01	Zn, Ca, P, B, Mn, Mo, Cr, Fe, Co, Rb, Sr
2	10.42	Sn, Pb, Cu, Si, S
3	5.16	Ag, U, Sb, Ca, V, Cd

2-4-2 SOIL GEOCHEMISTRY

The B-horizon soils (podzols) are largely sandy to coarse-grained and pale to dark brown in color. The <250 μm size fraction is acidic (pH varies from 4.2 to 4.9) and the organic matter content is between 2.1 and 4.8%. The dominant minerals in the soil include albite (68%), quartz (17%), low Fe muscovite (6%), and smaller amounts of hornblende-Fe (Nyade et al., 2013). Approximately 87% of the assay data had U concentrations below the detection limit of 0.1 ppm. In all, four samples recorded U concentrations in the range of 100-405 ppm U, 13 samples recorded concentrations that range between 20 and 100 ppm U, and 29 samples have U concentrations between 10 and 20 ppm U. All element concentrations below analytical detection limits were set to 0.05 ppm, essentially half the detection limit to allow for parametric statistical analysis and spatial presentation of the geochemical data (Grunsky, 2010).

Figure 9 shows a spatial association of U concentration in the <250 μm fraction of B-horizon soil and the radiometric U/Th signatures in the southern part of the study area. The highest U concentrations (>100 ppm) occur in silt samples collected from areas in the immediate vicinity of a northeast-trending fault structure and proximal to a bog situated on the southeastern edge of the study area. Areas with elevated U concentration in the <250 μm size fraction of B-horizon soil coincide with margins of the detected radiometric U/Th anomaly (Fig. 2-7). A comparison of spatial plots of U concentrations in spruce twigs with uranium content in B-horizon soil highlights a southeasterly offset of soil anomalies and suggests a trail of lateral dispersion from the major anomaly defining the mid-western edge of the study area and proximal to the shoreline of Jacque's Lake.

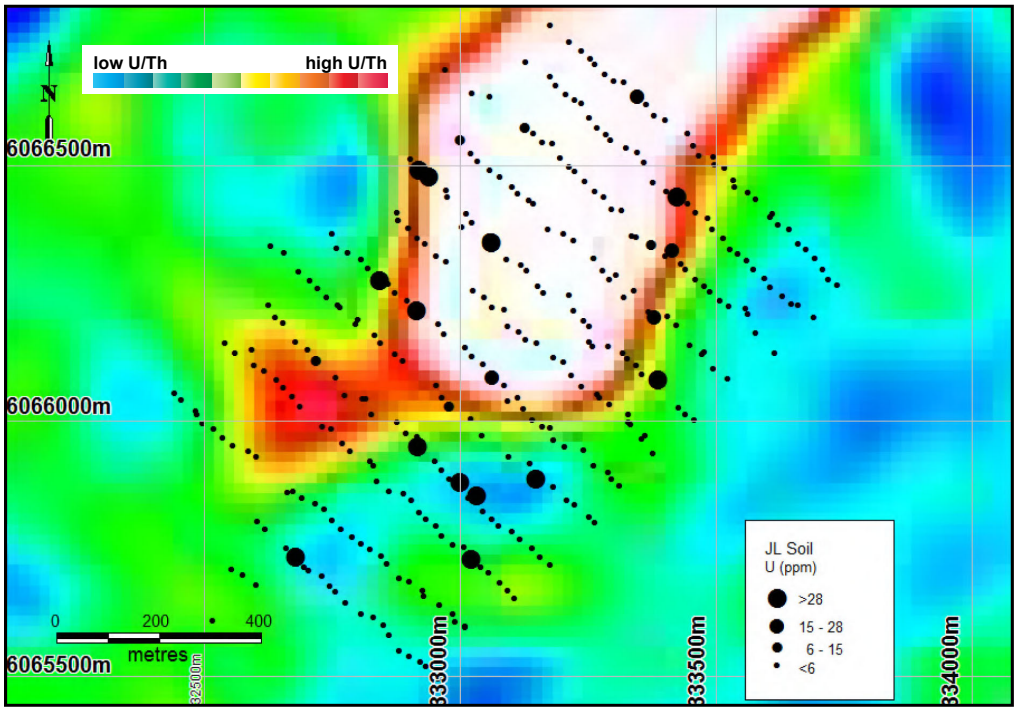

Figure 2-7: Dot plot of uranium enrichment in the < 250 µm size fraction of B-horizon soil sampled from the southern portion of the Jacque's Lake prospect, Central Mineral Belt, Labrador. [UTM NAD 83 Zone 21; MapInfo Professional version 10.0].

Binary plots of the soil geochemical data display weak associations of U with Pb, V, Sr, and Mo (Fig. 2-8). Factor analysis of the soil geochemical data illustrate interesting relationships between Cu, Mo, and Pb, and Mn, Zn, V, Co, P, and Fe. Soil samples collected from the southeastern part of the study area define significant contrasts for V, Pb, and Ag against the background concentrations and correlated well with the detected U/Th radiometric signatures. To the contrary, plots of Mn, Zn, V, Co, P, and Fe produced erratic patterns with point anomalies outside the elongate northeast trail of radiometric U/Th anomaly. Although mineral assemblages containing pyrite, chalcopyrite, galena,

sphalerite, and molybdenite have been reported in association with U mineralization at the Jacque's Lake area (Wilton, 1996; Cunningham-Dunlop and Lee, 2008), elements including Fe, Cu, Mo, and Zn are poorly correlated with U abundance in the <250 μm size fraction of B-horizon soils.

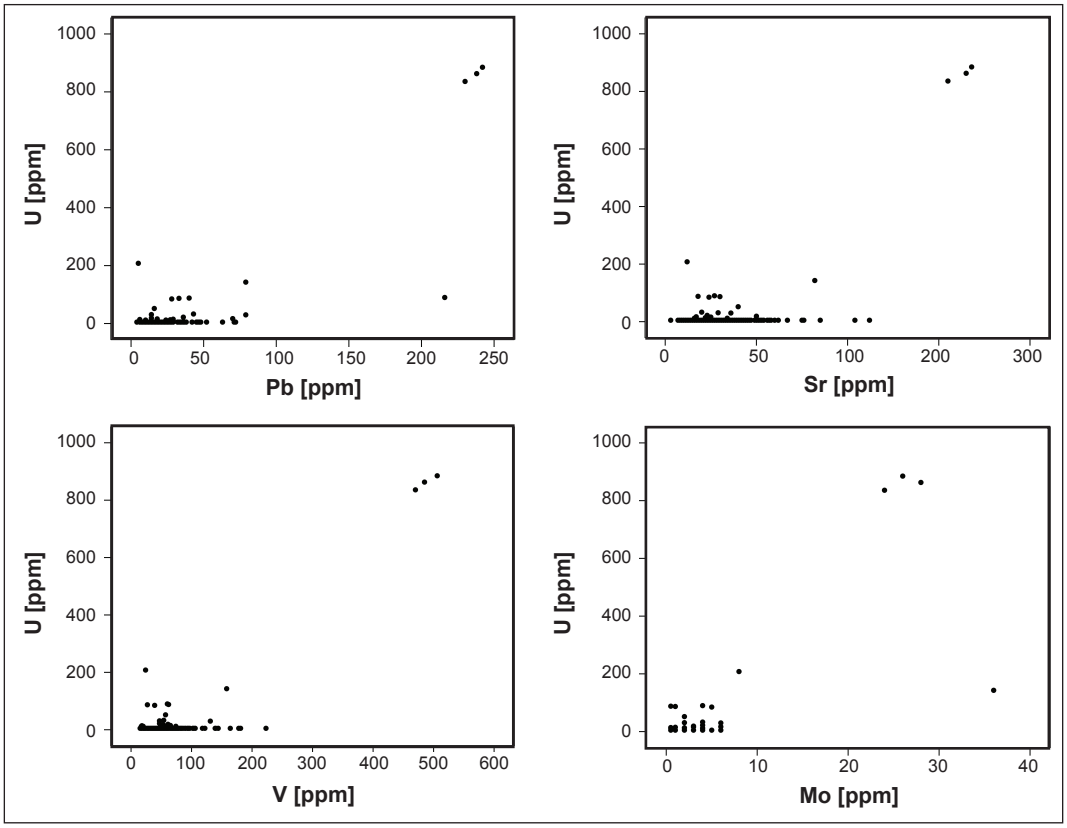

Figure 2-8: Binary plots for U versus Pb, Sr, V, and Mo in the <250 μm size fraction of soil collected from the southern part of the Jacque's Lake area, Central Mineral Belt, Labrador.

2-5 DISCUSSION

The discovery of uranium near Makkovik in the 1950s and 1960s led to intense exploration in eastern Labrador (Kerr and Sparkes, 2009). Initial exploration was based on rudimentary techniques such as boulder tracing and airborne radiometric surveys which defined U showings in areas with abundant outcrops and thin Quaternary glacial till cover (Gandhi, 1978). Radiometric surveys, though useful for detecting mineral anomalies, have limited effective depth penetration through cover greater than 30 cm. Therefore, even a few metres of glacial till can effectively mask buried mineralization.

The radiometric survey undertaken by Aurora Energy Ltd. on the Jacque's Lake property defined strong bedrock radiometric U/Th anomalies, which appear to be spatially coincident to a major northeasterly-trending fault. Brinex made similar observations in a boulder tracing campaign of 1967 (Gandhi, 1978). Prominent radiometric U/Th anomalies were noted to define a northeast-southwest trend of approximately 2 km x 8 km and are underlain by strata of the Aillik Group. Minor offsets along the northeast-southwest radiometric U/Th anomaly are attributed to easterly-oriented cross faulting, suggesting that the radiometric anomaly is not simply a glacial dispersal fan of mineralization float. The spherical anomalies that define the southern zone could be attributed to upward effusions of element-rich groundwater through fractures and lineaments that characterize this southern part of the prospect because the soil is directly overlain on the bedrock. The radiometric anomalies are interpreted to reflect structurally-controlled bedrock-hosted uranium mineralization that has been enhanced by a combination of upward migration of U-bearing groundwaters along the major southeasterly-trending fault into the surface soil.

The derived biogeochemical data for black spruce twigs, and barks, showed evidence of high enrichment of U and related pathfinder elements in areas where the mineralized bedrock is concealed by extensive glacial till cover. The plant chemistry, in addition, highlighted significant differences in levels of metal accumulation in different parts of the plant (i.e. twigs, stem, bark, and needles/leaves), suggesting that not all plant materials are equally useful as biogeochemical sampling media. Elements including Al, Mg, Rb, Zn, Mn, and Cd were highly enriched in spruce bark.

Factor analysis of the spruce twig biogeochemical data discriminated the elements based on their geochemical relationships. In this study, the first factor discriminates major and minor plant nutrients (e.g. Zn, Ca, P, B, Mn, Mo, Cr, Fe, Co, Rb, and Sr) (Fig. 2-8) and invariably reflects the health of the spruce trees (Silva and Uchida, 2000; Kabata-Pendias and Pendias, 2001; Dunn, 2007a). Rubidium and Sr are nonessential plant nutrients; nonetheless, they can respectively act as nontoxic substitutes for K and Ca, due to similarities in their chemical properties (Kabata-Pendias and Pendias, 2001). The grouping of Sn, Pb and Cu in the second factor suggests the occurrence of these elements as labile species liberated from their respective sulphide and lithophile host minerals. In other words Cu, Pb, and Sn were predominantly present as mobile species in the acidic soil pore water/mineral-rich groundwaters rather than as insoluble sulphides or silicates. The third factor comprising of Ag, U, Sb, Ca, and V constrained the buried uranium mineralization and related pathfinder elements.

Spruce twigs produced significant geochemical responses for U in areas directly over peat bogs and in the immediate vicinities of southeasterly fault structures through

uptake of labile metallic species in enriched bogs and groundwater. Peat bogs have the tendency to be enriched in U due to their capacity to sequester uranyl cations through complexation and cation exchange reactions (Owen and Otton, 1995). Variations observed in the magnitude of element accumulation in different plant parts were reported in other studies, in particular, Dunn (1981), Dunn et al. (1995), Chaudhuri et al. (2003), Anand et al. (2007), Nkoane et al. (2007), and Reid and Hill, (2010). Anand et al. (2007) reported marked differences in the levels of metallic element accumulation in litter, roots, bark, branch wood, and phyllodes of *Acacia aneura* collected from different locations in the Yilgarn Craton, Australia.

The elements Pb, V, Ca, Ag, Sb, Ce, and Be exhibit moderate to high correlations (i.e. Pearson correlation coefficients ranged between 0.49 and 0.95) with U in the spruce twig data and, therefore, are considered good pathfinders for buried U mineralization. Association of Pb with U could be a consequence of uptake of radiogenic Pb from the buried mineralization. Further, the elevated concentration of Ca and its strong association with U in the black spruce twigs could also indicate an uptake of labile Ca ions from the widespread calcite alteration reportedly associated with the uranium mineralization (Cunningham-Dunlop and Lee, 2008; Piercey, 2009).

Uranium signatures in the <250 μm size fraction of B-horizon soil produced a series of isolated and uncorrelated anomalies restricted to areas with thin glacial till cover. The anomalies are easterly oriented and appear to be laterally displaced relative to anomaly patterns defined by spruce twig and the radiometric U/Th anomalies. This pattern of anomalies suggests that a subvertical migration of labile metal ions may have

occurred from the buried U mineralization through the overburden material. A subvertical migration indicates that surface geochemical haloes will be displaced from buried ore (Dunn, 1981). Thus, the surface anomalies may be displaced to one side or may produce rabbit ear anomalies if displaced to opposite sides of the buried ore-body (Dunn 1981). Authors, including Hamilton (1998), Hall et al. (2004), and Aspandia et al. (2008) have presented extensive reviews of trace metal dispersal mechanisms through overburden materials.

2-6 CONCLUSIONS

The multi-faceted approach using airborne radiometric, soil geochemistry, and analysis of vegetation produced good results in delineating zones of anomalous uranium and related element concentrations in areas covered by glacial sediments. The radiometric survey outlined a well-defined northeast-southwest linear signature of the buried uranium mineralization. Areas with peak U anomalies were associated with folded strata of elevated U/Th signatures over a distance of approximately 2 km. However, the radiometric responses were weak or absent in valleys filled with glaciofluvial sediments, especially, in areas adjacent to the shores of Jacque's Lake. Spatial plots of uranium content in spruce twigs and bark delineated zones of mineralization and also exhibited good spatial association with the detected U/Th radiometric anomaly. Conversely, association between U signatures in the sub 250 μm size fraction and the radiometric responses was rather weak. Thus, the sub 250 μm size fraction of B-horizon soils generally exhibited discrete patterns of U enrichment, but provided minimal information

about the underlying mineralization. The highest U concentrations occurred in silt samples collected from the southern portion of the study area and are laterally displaced southeast of the detected norheast-southwest radiometric anomaly.

Enrichment of metallic elements was generally low in the ash of Labrador tea stems and leaves, and in spruce needles. Dunn (2007a) attributed the low enrichment in spruce needles to a barrier mechanism. The ash of spruce twigs provided the greatest anomaly-to-background contrasts for U and the pathfinder elements (Pb, Ag, Sb, Ce, Be, Tl, and Ca) and well defined multi-element haloes over the mineralised zones. Uranium content in the bark of spruce trees outlined the buried mineralization in spite of the relatively low enrichment of metallic elements. The biogeochemical responses generally outlined the buried mineralizations more precisely than B-horizon soils and thus provided adequate information for further geological and geochemical investigations and for locating drilling targets in subsequent surveys.

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References

Agriculture Canada, (1998). The Canadian system of soil classification. 3rd ed., [online]. Available from http://sis.agr.gc.ca/cansis/taxa/cssc3/intro.html. [Cited 21 August 2009].

Anand, R.R., Cornelius, M., and Phang, C. (2007). Use of vegetation and soil in mineral exploration in areas of transported overburden, Yilgarn Craton, Western Australia: a contribution towards understanding metals transportation processes. Geochemistry: Exploration, Environment, Analysis, 7: 267-288.

Arne, D.C., Stott J.E., and Waldron, H.M., (1999). Biogeochemistry of the Ballarat East goldfield, Victoria, Australia. Journal of Geochemical Exploration, 67: 1-14.

Aspandia, M.F., Anand, R.R., and Gray, D.J. (2008). Geochemical dispersion mechanisms through transported cover: implication for mineral exploration in Australia. Open File Report 246. CRM LEME, Bentley, WA 6102.

Bailey, D.G., (1977). Geology of the Walker-MacLean Lake area, 13K/9E, 13J/12, Central Mineral Belt, Labrador; Government of Newfoundland and Labrador, Department of Mines and Energy, Mineral Division, Report 78-3

Batterson, M.J., and Taylor, D.M. (2004). Till geochemistry of the Melody and Moran Lake areas, Central Mineral Belt, Labrador (NTS map sheets 13J/12, 13J/7, 13K/9, 13K/10). Department of natural Resources, Geological Survey, St John's, Open File LAB 1392.

Batterson, M.J., and Taylor, D.M. (2005). Till geochemistry of the Lititial Lake area, Central Labrador. Department of Natural Resources, Geological Survey, Open File 13L/0121.

Brooks, R.R. (1983). Biological methods of prospecting for minerals. John Wiley & Sons, NY.

Brooks, R.R. (1972). Geobotany and biogeochemistry in mineral exploration. Harper and Row, NY.

Brooks, R.R., Dunn, C.E., and Hall, G.E.M. (1995). Biological systems in mineral exploration and processing. Ellis Horwood Limited, Hertfordshire.

Cannon, H.L. (1961). Botanical prospecting for ore. Virginia Minerals 7(1): 1-11.

Chaudhuri, D., Tripathy, S., Veeresh, H., Powell, M.A. and Hart, B.R. (2003). Mobility and availability of selected heavy metals in coal ash and sewage sludge-amended acid soil. Environmental Geology, 44: 419-432.

Cole, M.M. (1991). Remote sensing, geobotany and biogeochemistry detection of Thalanga Zn-Pb-Cu deposit near Charters Towers, Queensland, Australia. Transaction of Institution of Mining and Metallurgy, Section B: Applied Earth Sciences, 100: B1-B8.

Cunningham-Dunlop, I., Barbour, D. Buschman, A., Howson, S. King, M., Lennox-King. M., Trevors, M., and Valenta, R. (2005). Assessment report on geological, geophysical, geochemical and diamond drilling investigation on the CMB Project,

Labrador, Canada. Aurora Energy Inc., Altius Resources Inc., and Fronteer Development Group Inc.

Cunningham-Dunlope, I. and Lee, C. (2008). An update on the exploration activities of Aurora Energy Resources Inc. on the CMB uranium property, Canada, during the period January – December, 2007; Part II - CMB Mineral Resources.

Dunn, C.E. (1981). The biogeochemical expression of deeply buried uranium mineralization in Saskatchewan, Canada. Journal of Geochemical Exploration, 15: 437-452.

Dunn, C.E., Spirito, W.A., and Balme, R.G. (1995). Biogeochemical survey, Baie Verte area, Newfoundland: black spruce twigs (parts of NTS 12H/16 and 12I/1), Open File 2951. Geological Survey of Canada, St John's, NL.

Dunn, C.E. (2007a). Biogeochemistry in mineral exploration. Handbook of Exploration and Environmental Geochemistry, Volume 9. Elsevier.

Dunn, C.E. (2007b). New perspectives on biogeochemical exploration. *In* Milkereit, 2007. Proceedings of Exploration 07: Fifth Decenial International Conference on Mineral Exploration, pp.249-261.

Dye, P.J. (1996). Response of *Eucalyptus grandis* to soil water deficits. *In* Geochemical dispersion mechanisms through transported cover: implication for mineral exploration in Australia. Edited by M.F. Aspandia, R.R. Anand, and D.J. Gray 2008. CRM LEME Open File Report 246. Tree Physiology, 16: 233 – 238.

Gandhi, S.S. (1978). Geological setting and genetic aspects of uranium occurrences in the Kaipokok Bay-Big River area, Labrador. Economic Geology, 73: 1492-1522.

Grunsky, E.C. (2010). The interpretation of geochemical survey data. Geochemistry: Exploration, Environment Analysis, 10: 27-74.

Hall, G.E.M., Hamilton S.M., McClenaghan, M.B., and Cameron, E.M. (2004). Secondary geochemical signatures in glaciated terrain – Extended Abstracts. SEG 2004 – Predictive Mineral Discovery Under Cover Symposium, 33. University of Western Australia, Perth.

Hamilton, S.M. (1998). Electrochemical mass transport in overburden: a new model to account for the formation of selective leach geochemical anomalies in glacial terrain. Journal of Geochemical Exploration, 63: 155-172.

Hinchey, A.M., and LaFlamme, C. (2009). The Paleoproterozoic volcano-sedimentary rocks of the Aillik Group and associated plutonic suites of the Aillik Domain, Makkovik Province, Labrador (NTS Map Area 13J/14). Newfoundland and Labrador Department of Natural Resources, Report 09-1, pp.159-182.

Hulme, K.A. and Hill, S.M. (2003). River red gums as a biogeochemical sampling medium in mineral exploration and environmental chemistry programs in the Curnamona Craton and adjacent regions in NSW and SA. Advances in Regolith 2003, pp.205-210.

Ives, J.D. (1978). The maximum extent of Laurentide Ice Sheet along the east cost of North America during the last glaciation. Arctic, 31: 24-53.

Kabata-Pendias, A. and Pendias, H. (2001). Trace elements in soils and plants. 3rd Ed., CRC Press LLC, Boca Raton.

Kerr, A. and Sparkes, G.W. (2009). Mineral commodities of Newfoundland and Labrador. Geological Survey Mineral Commodities Series, 5: 1-12.

Lintern, M.J., Butt, C.R.M, and Scott, K.M. (1997). Gold in vegetation and soil – three case studies from the goldfields of southern Australia. Journal of Geochemical Exploration, 58: 1-14.

Liverman, D. Boger, R., Honarvar, P. and Vatcher, P. (1993). Till geochemistry, Cavers Lake and Hollinger Lake area (NTS 23J/9 and 16) western Labrador. Newfoundland Department of Mines and Energy, Geological Survey, Open File 023J/0303.

McCuaig, S.J. and Smith, J.J. (2005). Quaternary geology of the Snegamook Lake area, Central Mineral Belt, Labrador. *In* Current Research, Geological Survey of Canada, Report 05-1: pp.15-25

Nkoane, B.B.M., Wibetoe, G., Lund, W., and Torto, N. (2007). A multi-element study on metallophytes from mineralized areas in Botswana using ICP-AES and ICP-MS. Geochemistry: Exploration, Environment, Analysis, 7: 49-56.

Nyade, P.K., Wilton, D.H., Longerich, H.P., Thompson, G. and McNeill, P. (2013). Evaluation of uranium accumulation in black spruce trees. Research Journal of Environmental and Earth Sciences, 5(3): 113-122.

Owen, D.E. and Otton ,J.K. (1995). Mountain wetlands: efficiency uranium filters – potential impacts. Ecological Engineering, 5: 77-93.

Piercey, S.J. (2009). Preliminary evaluation of drill core geochemical data from the Jacque's Lake uranium deposit, Central Mineral Belt, Labrador. A technical report

for Aurora Energy Ltd., St John's, NL, Canada [online]. Available from http://aurora-energy.ca/reports.html [Cited 13 December 2011].

Reid, N. and Hill S.M. (2010). Biogeochemical sampling for mineral exploration in arid terrains: Tanami Gold Province, Australia. Journal of Geochemical Exploration, 104: 105-117.

Reid, N., Hill, S.M. and Lewis, D.M. (2008). Spinifex biogeochemical expression of buried gold mineralization: the great mineral exploration penetrator of transported regolith, Applied Geochemistry, 123: 76-84.

Ryan, G.A. (1989). Native trees and shrubs of Newfoundland and Labrador. Parks Division, Department of Environment and Lands, Province of Newfoundland and Labrador. St John's.

Silva J.A. and Uchida R. (2000). Plant nutrient management in Hawaii's soils, approaches for tropical and substropical agriculture. College of Tropical Agriculture and Human Resources, University of Hawaii, Manoa.

Stevenson, F.J. (1994). Humus chemistry: genesis, composition, reactions. 2nd ed., John Wiley & Sons. Inc, New York.

Wilton, D.H. (1996). Metallogeny of the Central Mineral Belt and adjacent Archean basement, Labrador. Mineral Resources Report 8, Government of Newfoundland and Labrador, Department of Miners and Energy, Geological Survey.

Appendix 2- 1: Element concentrations in randomly selected twig samples ignited at 450 ^{o}C

Sample		L5-16	L2-3	L7-8	L3-11	L9-2	L6-1	L10-6	L5-9	L7-9	L7-7	L6-13
Li	ppm	3.61	4.91	4.48	1.75	10.72	7.14	5.07	7.17	18.09	3.55	10.13
B	ppm	7.13	9.20	6.51	11.52	12.68	11.83	10.71	15.18	10.37	10.29	11.94
Mg	ppm	942.89	1427.70	1065.02	1139.19	1560.82	1145.44	830.56	671.83	1236.33	940.67	991.68
Al	ppm	94.97	107.96	99.35	77.24	77.98	79.41	73.15	82.41	95.91	68.41	98.77
P	ppm	645.30	876.28	821.87	1181.53	1254.70	841.10	1046.93	865.08	1210.20	805.51	930.00
Ca	ppm	2305.97	2565.68	1881.31	2228.76	2670.48	2705.65	1783.32	3399.09	2027.36	1939.99	1558.05
Ti	ppm	7.75	6.81	7.05	18.11	12.71	10.07	15.53	11.70	8.39	4.63	6.92
V	ppm	0.03	0.03	0.03	<0.001	<0.001	0.02	<0.001	0.05	<0.001	0.03	0.04
Cr	ppm	2.44	1.05	1.25	2.40	2.79	0.99	1.48	1.46	1.16	1.39	1.34
Mn	ppm	247.73	308.68	430.93	399.88	384.98	410.55	422.80	236.09	525.00	337.82	205.00
Fe	ppm	74.81	79.03	63.74	83.71	57.33	40.71	52.70	38.17	31.40	170.46	<DL
Co	ppm	1.00	0.56	0.43	6.48	0.59	0.50	1.50	0.61	0.95	0.74	0.42
Ni	ppm	1.57	3.45	1.25	3.51	25.97	1.79	4.29	3.30	2.49	3.38	1.72
Cu	ppm	3.31	5.31	3.42	5.22	4.73	4.11	8.60	4.29	3.82	4.02	3.36
Zn	ppm	36.54	215.01	35.57	32.94	36.46	32.59	41.81	59.06	51.12	41.17	36.73
As	ppm	0.43	<DL	0.24	<DL	0.50	0.29	1.63	0.48	0.89	0.00	0.37
Rb	ppm	9.79	8.92	9.50	14.88	17.65	14.81	18.64	26.51	10.70	9.16	15.32
Sr	ppm	13.02	6.04	11.62	5.64	6.56	5.98	5.37	8.12	3.27	5.99	4.36
Mo	ppm	0.15	<0.002	<0.002	0.19	0.20	<DL	0.11	0.16	0.09	0.13	<0.002
Ag	ppm	<DL	11.35	9.12	13.52	14.13	<DL	0.30	3.95	<0.001	<0.001L	<0.001
Cd	ppm	0.19	0.15	0.00	0.00	0.00	0.10	0.26	0.09	0.08	0.45	0.00
Sn	ppm	1.21	0.73	0.97	1.48	0.76	0.76	1.90	1.19	1.04	1.62	1.38
Cs	ppm	0.18	0.28	0.12	0.14	0.19	0.32	0.27	0.29	0.15	0.20	0.12
Ba	ppm	23.84	9.24	27.49	7.48	12.70	18.01	7.47	33.31	9.56	13.83	3.71
La	ppm	0.03	<0.001	0.02	0.02	0.03	0.01	0.02	0.02	0.07	0.02	0.02
Ce	ppm	0.07	0.04	0.04	0.06	0.06	0.04	0.04	0.05	0.14	0.05	0.04
Hg	ppm	0.08	0.19	0.23	0.27	0.03	0.02	0.21	0.23	0.14	0.07	0.20
Tl	ppm	0.02	0.03	0.02	0.02	<0.001	0.02	<0.001	<0.001	0.02	0.03	<0.001
Pb	ppm	0.41	0.32	0.26	0.33	0.51	0.24	0.37	0.23	0.23	0.53	0.34
Bi	ppm	0.07	0.07	1.98	2.88	0.07	0.01	3.17	0.07	0.07	0.07	0.07
U	ppm	3.96	0.01	2.42	3.56	6.43	2.02	4.71	<DL	2.66	1.93	<DL

Appendix 2- 2: Element concentrations in randomly selected black spruce twigs digested using HNO_3/HF (1:1) + drops H_2O_2

Sample		L5-16	L2-3	L7-8	L3-11	L9-2	L6-1	L10-6	L5-9	L7-9	L7-7	L6-13
Li	ppm	3.84	5.33	5.57	1.36	12.42	9.90	5.59	8.90	21.13	3.59	11.75
B	ppm	10.99	10.70	10.81	12.95	14.34	12.26	12.21	16.29	15.00	9.23	14.65
Mg	ppm	1204.58	1342.82	1257.72	1227.99	1821.10	1331.72	905.66	934.08	1432.52	1041.58	1159.18
Al	ppm	106.05	91.15	114.03	58.83	104.71	107.80	81.56	106.96	150.26	64.84	119.71
P	ppm	832.44	820.70	961.47	1304.76	1462.59	977.46	1136.96	1042.67	1381.09	873.04	1088.10
Ca	ppm	3024.19	2454.94	2480.00	2601.91	3138.85	3265.77	2016.93	4183.45	2548.40	1970.88	1638.95
Ti	ppm	9.52	5.90	7.56	19.30	14.88	14.31	16.06	14.01	11.01	5.00	8.98
V	ppm	0.07	0.05	0.05	<0.001	0.14	<0.001	0.03	0.01	<0.001	0.07	0.27
Cr	ppm	1.86	1.24	1.15	2.57	3.43	1.13	1.56	1.76	1.29	1.24	1.43
Mn	ppm	315.12	289.56	506.99	432.65	444.89	491.63	457.41	286.26	619.52	388.47	240.99
Fe	ppm	70.05	33.55	49.17	57.57	63.88	30.63	50.22	55.08	53.10	202.19	44.16
Co	ppm	1.26	0.47	0.47	6.92	0.75	0.56	1.55	0.73	1.04	0.68	0.55
Ni	ppm	1.99	2.28	1.21	1.95	3.08	1.57	1.47	3.06	2.80	2.49	1.47
Cu	ppm	3.80	4.14	3.71	4.79	4.33	4.33	4.76	4.52	3.87	3.45	3.73
Zn	ppm	40.36	39.31	37.70	31.77	38.28	34.78	26.43	66.03	26.06	34.88	32.24
As	ppm	0.30	0.26	0.49	0.14	1.01	0.58	1.71	0.57	1.19	0.22	0.60
Rb	ppm	12.35	8.48	11.41	16.24	20.67	18.21	19.76	32.31	12.74	10.32	18.52
Sr	ppm	15.98	5.65	14.14	6.11	7.76	7.04	5.75	9.91	3.84	6.55	5.07
Mo	ppm	0.17	0.09	0.11	0.25	0.46	0.14	0.12	0.20	0.09	0.11	0.14
Ag	ppm	<0.001	12.00	7.57	9.20	10.39	<0.001	<0.001	3.37	<0.001	<0.001	<0.001
Cd	ppm	0.05	0.17	0.07	0.17	0.09	0.02	0.06	0.07	0.01	0.01	0.01
Sn	ppm	1.31	0.70	1.22	1.61	0.94	0.94	2.01	1.44	1.35	1.84	1.75
Cs	ppm	0.20	0.26	0.16	0.16	0.23	0.39	0.27	0.34	0.19	0.20	0.13
Ba	ppm	30.60	8.96	32.91	8.06	14.36	21.91	7.99	40.95	11.56	19.18	4.32
La	ppm	0.04	0.01	0.02	0.02	0.03	0.02	0.03	0.04	0.08	0.02	0.02
Ce	ppm	0.09	0.03	0.06	0.05	0.05	0.04	0.05	0.06	0.18	0.03	0.04
Hg	ppm	0.37	0.59	0.51	0.71	0.10	0.16	0.56	0.54	0.49	0.32	0.66
Tl	ppm	0.02	0.04	0.02	0.02	0.02	0.04	0.01	0.01	0.02	0.04	0.02
Pb	ppm	0.47	0.36	0.29	0.30	0.65	0.27	0.24	0.28	0.28	0.54	0.33
Bi	ppm	<0.001	<0.001	2.07	1.94	1.61	<0.001	1.82	<0.001	<0.001	<0.001	<0.001
U	ppm	3.76	<0.01	3.68	3.07	6.65	2.29	4.83	<0.01	2.26	2.12	<0.01

Appendix 2- 3: Element recovery in standard reference materials

Elements	SRM-1575		SRM-1547		CLV-1		CLV-2	
	Measured	Certified value	Measured	Certified value	Measured	Certified value	Measured	Certified value
Li	7.30		8.35		8.88		10.69	
B	13.13		28.19	29	14.10		38.56	
Mg	1049.98		0.44	0.432	1171.06		736.62	
Al	534.40	545	286.13	249	1309.55	1430	348.13	320
P*	0.11	0.12	1436.83		<DL		<DL	
Ca*	0.39	0.41	1.62	1.56	0.60	0.63	0.73	0.77
Ti	24.26		37.28		105.24		22.20	
V	0.33		0.35	0.37	2.94	3.6	0.34	0.76
Cr	1.67	2.6	1.00	1	<DL		<DL	
Mn	608.09	675	97.50	98	526.95	612	1765.31	1940
Fe	193.91	200	271.74	220*	1859.62		294.58	
Co	0.08	0.1*		0.07	0.48		<DL	
Ni	2.39	3.5*	1.33	0.69	3.13		0.84	
Cu	2.42	3	2.34	3.7	4.76		1.70	
Zn	61.72		29.11	17.9	75.03		61.94	
As	0.08	0.21	0.74	0.06	4.88		0.45	
Se	<DL		0.05	0.12	<DL		<DL	
Rb	11.17	11.7	17.49	7*	4.08		3.34	
Sr	4.33	4.4	56.93	53	30.22		18.16	
Mo	<DL		0.10	0.06	2.20		0.08	
Ag	0.03		0.02		0.09		0.02	
Cd	0.16	<0.5*	0.02	0.03*	0.19			
Sn	1.16		10.32		1.62		1.17	
Sb	0.18	0.2*	0.06	0.02*	0.20		0.04	
Cs	0.12		0.07		0.08		0.03	
Ba	6.28		123.99	124	56.91		23.98	
La	0.12	0.2*	9.59	9*	1.13		0.30	
Ce	0.24	0.4*	10.91	10*	2.40		0.67	
Hg	0.03	0.15		0.037				
Tl	0.04		0.01		0.03		0.02	
Pb	10.22	10.8	0.57	0.87	9.51		1.16	
Bi	0.02				0.07			
U	0.02	0.02	0.02	0.015*	83.0	86.8	3.20	3.6

All element concentrations in ppm
*wt (%)

Appendix 2- 4: Element concentrations in the <250 μm sized-fraction of B-horizon soil

	B-horizon soil	
Element	Median	Maximum
Ag	0.1	1.2
Al (%)	0.61	3.04
As	11	53
B	0.9	2.1
Ba	31	268
Be	0.25	6.3
Bi	1	6
Ca (%)	0.44	3.47
Cd	0.25	0.7
Co	7	34
Cr	41	667
Cs	<0.01	<0.01
Cu	6	120
Fe (%)	1.94	7.51
Ga	5	10
Hf	ND	ND
Hg	0.5	5
K (%)	0.04	0.95
La	15	218
Mg (%)	0.27	3
Mn	176	1830
Mo	2	26
Ni	12	239
P (%)	0.023	0.104
Pb	16	242
S (%)	0.01	0.36
Sb	1	8
Sc	3	11
Sr	25	168
Ti (%)	0.24	0.54
Tl	5	27
U	5	885
V	52	506
W	0.3	5
Zn	29	251

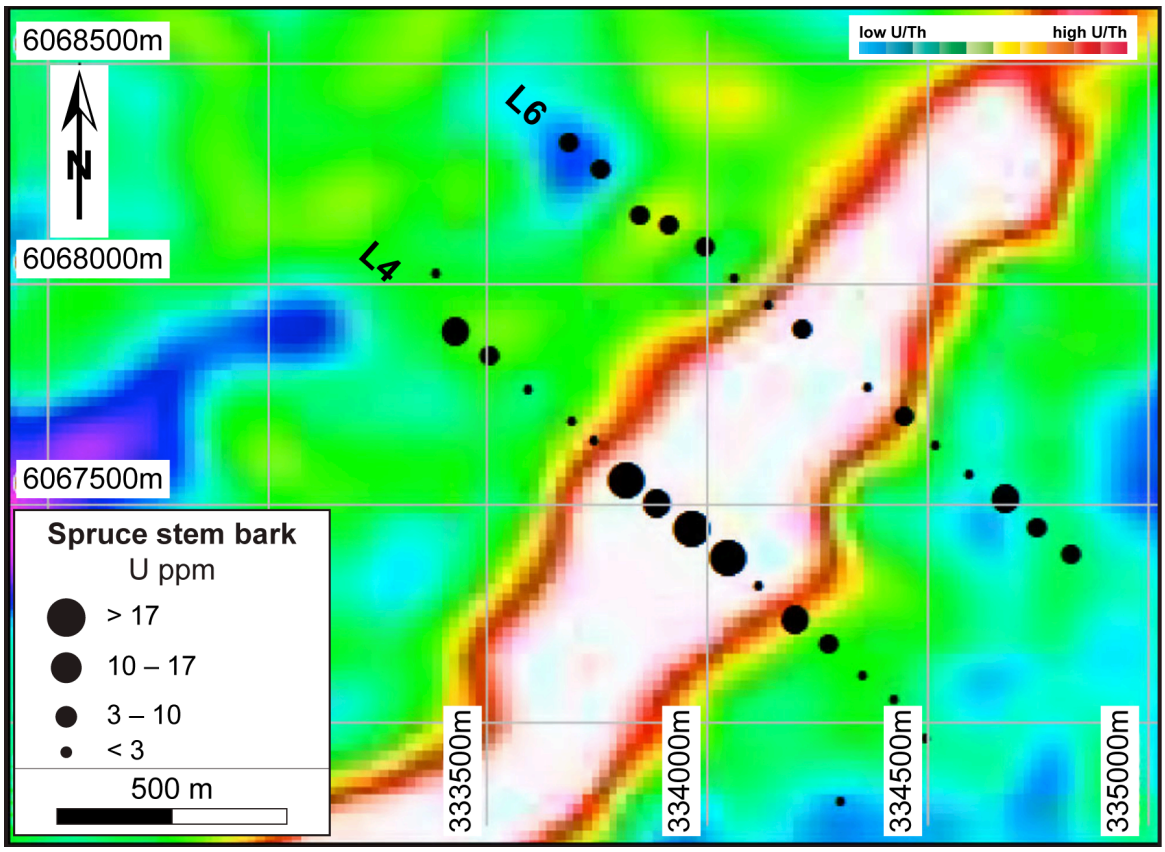
ND = not detected; All concentrations in ppm unless otherwise stated

Appendix 2- 5: Aerial view of typical vegetation cover in the Jacque's Lake area of the CMB

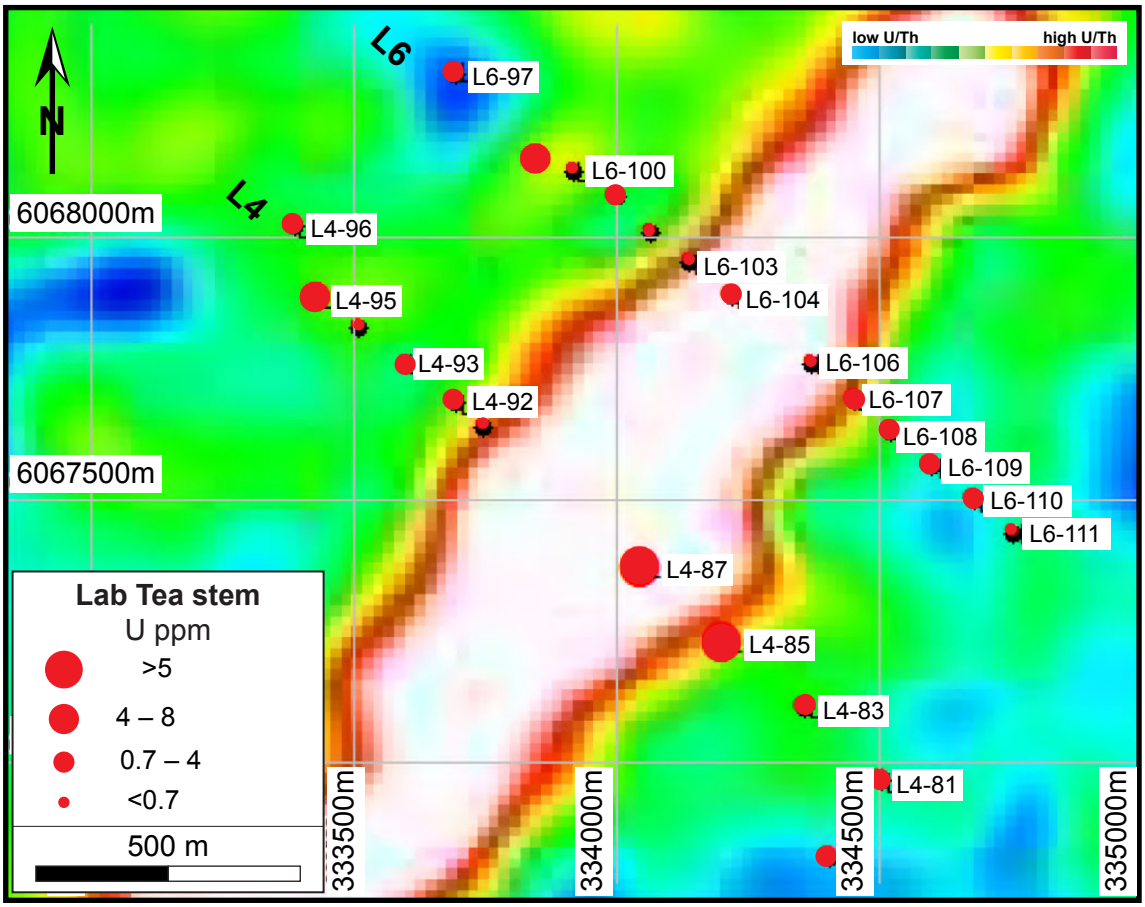
Appendix 2- 6: Airborne sampling of approximately 7-10 year old twigs from the extremities of black spruce trees located on the Jacque's Lake prospect

Appendix 2- 7: Sampling of the outer bark of a black spruce tree stem (photograph of Drs. Wilton and Thompson sampling the Voisey Bay area, Labrador)

Appendix 2- 8: Spatial plot of U concentrations in black spruce stem bark sampled along two NE-SW traverses crosscutting the detected U/Th radiometric anomaly



Appendix 2- 9: Spatial plot of U concentrations in the stems of Labrador tea sampled along two traverses crosscutting the NE oriented radiometric anomaly



Chapter 3

In

Praise Nyade, Derek Wilton, Henry Longerich, Gary Thompson, Paul McNeill (2013). Evaluation of uranium uptake in black spruce trees. Research Journal of Environmental and Earth Sciences, vol. 5, No. 3, p. 113-122. 8155-RJEES-doi

EVALUATION OF URANIUM ACCUMULATION IN BLACK SPRUCE TREES

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Abstract

This study evaluated the metal accumulation capacity of black spruce seedlings (*Picea mariana*) with a special focus on uranium and compared uptake from peat and podzol soil treated with various concentrations of uranyl nitrate hexahydrate. Bioavailable indices for uranium in the substrates, accumulation factors, and transfer factors for the translocation of U between different plant organs were estimated. The results showed a higher concentration of U in shoots with accumulation factors up to seven times greater than values determined in roots. Uranium accumulation in stems was several orders of magnitude higher than the metal content in roots and needles. Transfer from the substrate to the plants was influenced by substrate-specific properties, resulting in a higher uptake of U from soil than from peat. The pattern of U accumulation was consistent with that previously reported in field studies. Metal accumulation values also displayed a linear progression with bioavailable metal concentrations in the substrate, suggesting that black

spruce trees can be classified as bioindicators of uranium content in underlying substrates rather than hyperaccumulators of the metal.

3-1 INTRODUCTION

Multi-element analysis of vegetation has become a powerful tool for defining surficial chemical signatures of buried mineralisation as well as the environmental monitoring of anthropogenic inputs of pollutants (Market, 1993; Brooks, 1995; Dunn, 2007; Reid and Hill, 2010). Mineral exploration using conventional geochemical methods in glaciated and deeply weathered environments is often hampered by the significant depths of exogenous material, and hence, has a decreased potential to detect deeply buried ore (Anand et al., 2007). Some plant species growing in otherwise non-prospective areas, however, have developed the ability to accumulate massive amounts of the indigenous metals in their tissues, reflecting the underlying substrate chemistry without exhibiting symptoms of toxicity (Baker and Brooks, 1989). These plants highlight the buried geological features through interactions and the uptake of metallic elements from the many cubic meters of soil, bedrock, and groundwater that surround the roots.

Coniferous trees from the genera, *Picea, Pinus,* and *Albies* (Dunn, 2007); deciduous plants of the family, *Brassicaceae e.g. Arabidopsis halleri* and *Noccaea caerulescens* (Kramer, 2010); and shrubs, including mountain alder, Labrador tea, and aspen (Walker, 1979; Gordon 1992; Dunn, 2007) have been perceived as metal hyperaccumulators and consequently used as biogeochemical sampling media to detect

buried ores in various reconnaissance surveys in North America and Europe (Brooks et al., 1995; Dunn, 2007).

Non-prospecting applications of plants that utilize the ability of plant roots to take up and accumulate high concentrations of metallic elements from underlying substrates for environmental monitoring have gained wide acceptance (Markert, 1993; Brooks, 1995). Plants, including bryophytes and ferns (Kramer, 2010; Selinus, 1988, 1989) and vascular plants *e.g.* Labrador tea, dandelion (Gorden, 1992), and coniferous trees (*e.g*. Norway spruce) have been widely applied to monitor the metal contamination of soils (Gordon, 1992). Specifically, *Pinus sylvestris, Robinia pseudoacacia, Tilia cordata, Picea abies,* and *Acer platanoides* were variously used to monitor heavy metal (Pb, Cd, Hg, Zn, *etc.*) pollution from sites near point emission sources (Ozturk et al., 2008).

Black spruce trees have unique characteristics that make them of particular interest as biogeochemical sampling media for prospecting buried ore and environmental monitoring. The conifer has a broad ecological amplitude and is widely distributed across the boreal forests of North America, Europe, and northern Asia. Through its use in biogeochemical studies, black spruce has gained recognition as a metal hyperaccumulating plant (Dunn, 1981, 2007). Studies elsewhere, unpublished data from areas in Atlantic Canada in particular, have reported very low U enrichments in twigs, bark, and needles collected from known mineralised areas, thus creating uncertainties about the metal accumulating potential of spruce trees (Dunn C.E., personal communication). Notably, biogeochemical surveys from areas in Central Labrador returned low concentrations of metallic elements, including uranium in areas of known

bedrock mineralizations. It is these contrasting reports that necessitated the interest in investigating metal accumulation in spruce trees with a special focus on U.

The transfer of U from soil to plants has typically been studied in food crops/agricultural plants (*e.g.* Shahandeh and Hossner, 2002) and in hydroponic experiments using spiked nutrient solutions. Bioaccumulation and metal transfer factors from such studies may have limited validity for describing the behaviour of U in coniferous trees. Thus, experiments using nutrient solutions as growth media only provide approximate estimates of the processes occurring in the complex soil pore water under ordinary conditions and are remote from the real conditions existing in the field because soil and liquid media are very different systems. Uranium commonly exists in bioavailable forms in liquid media, whereas several factors affect its mobility and in particular, its availability to biota (Shtangeeva, 2008). The research described in this paper investigated uranium uptake and translocation to different parts of *Picea mariana*. Substrate properties and substrate-to-plant transfer of the metal and its distribution across plant parts were examined. The goals were: (1) to examine U uptake and distribution across different parts of plants grown on U-enriched soil and peat; (2) determine the bioaccumulation and transfer factors of U in spruce trees; and (3) investigate the substrate impact on the uptake/accumulation of the metal.

3-2 MATERIALS AND METHODS

3-2-1 REAGENTS AND MATERIALS

Acids used for sample preparation were analytical grade and included: nitric acid (70 % v/v), hydrochloric acid (38 % v/v), and certified A.C.S. ammonium acetate; all supplied by Fisher Scientific (Fair Lawn, NJ, USA). Uranyl nitrate hexahydrate was supplied by BDH Chemicals Ltd (Poole, England). A set of reference materials was included in each batch of sample preparations and analysis as a measure of precision, accuracy, quality assurance, and quality control.

The standard reference materials (SRMs) were comprised of SRM-2710a (Montana I soil), SRM-2711a (Montana II soil), SRM-1547 (peach leaves), and SRM-1575 (pine needles) from the National Institute of Standards and Technology (NIST) (Gaithersburg, USA). Vegetative radionuclide reference materials, CLV-1 (spruce twigs) and CLV-2 (spruce needles) were obtained from the Canadian Certified Reference Material Project, CANMET Mining and Mineral Sciences Laboratories, ON, Canada. Deionized water (nano-pure water, 18 mΩ cm) was procured from a Millipore Elix-5/Mill-Q water purification system (Bedford, ON). Two-year old black spruce plants were obtained from the plant nursery of the Forestry Division of the Department of Natural Resources, Province of Newfoundland and Labrador. Premier Horticulture Limited supplied Pro-mix potting peat.

3-2-2 ANALYSES OF SOIL AND PEAT

Bulk podzol soil collected from the Michelin uranium deposit, Central Mineral Belt, Labrador was used for pot experiments in a greenhouse study at the Memorial University Botanical Gardens, St. John's, NL, Canada. The soil, collected from depths of 5-15 cm, was shipped to the Department of Earth Sciences, MUN, where it was air-dried at room temperature, sieved through a 4 mm mesh, and stored at 4 ^{o}C until ready for use.

The total metal content in the <250 μm size fraction of soil was determined by HNO_3/HF (1:1) digestion of approximately 0.1 g sample followed by aqua regia. The resultant solutions were analyzed using an ICP-MS (ELAN DRC II, PerkinElmer Sciex, Concord, ON, Canada) for a suite of 36 elements. The mineral composition of <250 μm sized-fraction of soil was analyzed with a Mineral Liberation Analysis-Scanning Electron Microscope (MLA-SEM) using the method developed by Wilton and Winter (2012) (see Appendices 3-8 to 3-12). Reagent blanks were included to ensure that solutions were not contaminated with U or other analytes during sample preparation. Results are reported on a dry weight basis.

The organic matter content of the soil was estimated by ignition at 550 ^{o}C. The pH of the soil and peat were determined using 1:1 slurries of substrate and deionized water. The pH meter (model 310, Orion Research Inc., FL, USA) was calibrated prior to use and has an estimated uncertainty of ±0.05 pH units. Estimates of the available U fraction in the substrates dosed with uranyl nitrate and the experimental control substrates were obtained using a 1 mol/L ammonium acetate (NH_4Ac, buffered at pH 4.5) leach. The U

spiked and the experimental control peat samples were ignited at 550 ^{o}C and the ash was dissolved in nitric acid for analysis using ICP-MS.

The Tessier *et al*. (1979) method was applied to sequentially extract U sorbed onto various mineral phases in the substrates. A 0.5 g sample was sequentially subjected to a series of extractants, including 1.0 mol/L magnesium chloride for exchangeable metals; 1.0 mol/L sodium acetate for cations bound to carbonates; 0.04 mol/L hydroxylamine HCl in 25 % acetic acid for components bound to Fe-Mn oxide phases; 0.02 mol/L nitric acid and 5.0 M hydrogen peroxide for cations sorbed onto organic phases; and a 2:1 mixture of 8 mol/L nitric acid and 29 mol/L hydrofluoric acid for residual components. The sum of all the U fractions was compared with the total U determined in substrates subjected to HNO_3/HF (1:1) and aqua regia digestions.

3-2-3 PLANT CULTIVATION IN A GREENHOUSE

Pots filled with 500 g (oven dried) of the <4 mm fraction of bulk soil were mounted on trays and were treated with different doses of uranyl nitrate hexahydrate. The doses comprised of 100 mg /kg, 250 mg /kg, and 400 mg /kg. A second set of pots was filled with pro-mixed peat and was treated much the same away as soils. The substrates (peat and soil) were fertilized according to a fertilizer regime recommended by botanists at the MUN Botanical Gardens for black spruce seedlings. The substrate amendments (uranium nitrate hexahydrate and soil fertilizer) were dissolved in deionized water, though the quantities used were calculated on a dry weight basis.

Each dose treatment and control (non-amended soil and peat) were replicated ten times given a total of 100 pots. The pots were watered with deionized water (18 mΩ cm @ 25 ^{o}C) and the field capacity determined using a VH 400 Soil Moisture Sensor Probe connected to a Metex M-4650 digital multimeter (Metex Corporation, Seoul, South Korea). The two-year-old seedlings of *Picea mariana* were transplanted from potting compost into pots containing U-enriched substrates and the experimental control and were periodically watered in the trays. Watering of the potted seedlings through the trays was to minimize the possible contamination of stems and needles with U-enriched soil particles during aerial watering. Tray watering also minimizes leaching of metals from the potting substrates. Another set of samples was comprised of seedlings cultivated in non-amended soils and peat, and was treated monthly with a dose of 50 mL of 250 mg/kg uranyl nitrate solution. Plants were grown for 16 weeks in the greenhouse with a natural day light/night regime in the summer of 2011. At harvest, the aerial portions of the plants were removed just above the substrate (soil/peat) level using a pair of Teflon-coated shears, while roots were carefully extracted from the soil and washed copiously with tap water, followed by deionized water.

3-2-4 PLANT ANALYSES

Plant parts (roots, stem, and needles) were washed with deionized water after harvest. Roots, stems, and needles were oven-dried, pulverized in a cap mill, and accurately weighted samples of approximately 1.0 g were ignited at 450 ^{o}C, following which the ash was dissolved in HNO_3 for ICP-MS analysis.

3-2-5 STATISTICAL ANALYSES AND QUALITY CONTROL

Data were analyzed and presented as mean and standard deviation (X±SD) of ten replicates using SPSS statistical package (version 20 for Mac). Multiple comparisons of means were determined by the Tukey's test. Student's t-tests were used to detect significant differences between mean U concentrations in substrates and plant parts. The precision of analytical procedures was expressed as a relative standard deviation (RSD) calculated as the standard deviation divided by the mean. The RSD values for uranium in all samples ranged from 3-10 %. The recovery rates for U in the standard reference materials were within 90 % ± 10%. Table 3-1 shows uranium recovery in standard reference materials.

Table 3- 1: Uranium recovery in standard reference materials

Standard Reference Materials	Measured value (μg/g)	Certified value (μg/g)
NIST SRM 1547	0.012	0.015*
NIST SRM 1575	0.017	0.02
NIST SRM 2711a	2.66	3.01
NIST SRM 2710a	8.8	9.11
CLV-1	81	86.8
CLV-2	3.2	3.6

* recommended value

The Biological Accumulation Factor (BAF) was calculated as the ratio of metal content in shoots to the available metal concentration in the soil (Negri and Hinchman, 2000; Kidd et al., 2007); The Translocation Factor (TF) was defined as the ratio of the

metal content (mg/kg) in the plant stem to the concentration in the roots (Sheppard and Evendon, 1988; Ramaswami et al., 2001; Kidd et al., 2007; Al-Qahtani, 2012); and the Bioavailability Index (BI) is the ratio of available metal concentration (mg/kg) to the total metal concentration (mg/kg) in the soil (Kidd et al., 2007). All concentrations were computed on a dry weight basis.

3-3 RESULTS AND DISCUSSION

Podzol soil collected for the study was largely sandy, coarse grained, and light brown in color. Mineral liberation analyzer - scanning electron microprobe (MLA-SEM) analysis of the <250 μm fraction showed that the dominant mineral composition of the soil was albite (68%), quartz (17%), low Fe muscovite (6%), and with small amounts of hornblende (Wilton, 2012).

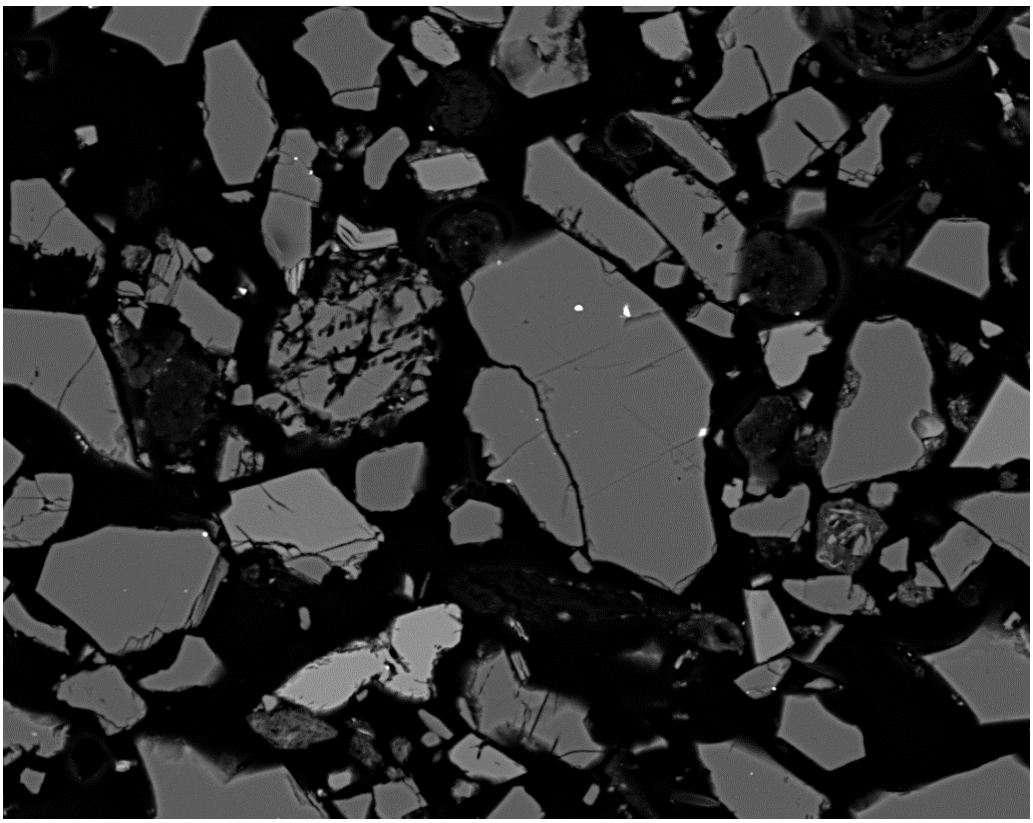


Figure 3- 1: Si_uranium particle (white) in albite host grains (grey) of untreated soil (Wilton, 2012).

The pH ranges determined in the soil and peat were 4.2-4.6 and 3.9-4.3, respectively. The detected low pH in both substrates promoted solubilization and increased the mobility of uranyl ions in substrate pore water as well as the speciation of U(VI) organic species (such as fulvic and humic acids) in peat. The pH of the treated substrates did not vary significantly over the period of stabilization, indicating that substrate amendments had no significant effect on pH. The organic matter content of the

soil ranged from 4.6 - 4.8 %. Table 3-2 shows the measured essential plant nutrients - S, P, Ca, and B and related physicochemical parameters in the soil and peat.

Table 3- 2: Plant nutrient composition in substrates and related physicochemical parameters

Parameter		Substrate	
		Soil	Peat
pH			
OM	%	4.6-4.8	85
N	% total	1.32	2.4
K	ppm	ND	ND
S	ppm	0.12	<0.01
P	ppm	1631.4	124
Ca	ppm	0.84	0.02
Zn	ppm	13	1.04
Cu	ppm	<0.01	0.3
B	%		0.4
Mo	ppm	3.56	0.012
Mn	%	0.05	0.0004
Moisture content	%	5.1	18

Table 3-3 illustrates the level of U fractionation in various mineral phases from the <250 μm fraction of non-treated (control) and amended soil. MLA-SEM analysis of non-amended soil using back scattered electron (BSE) image gray scale underscored the occurrence of uranium largely as uraninite particle inclusions in major minerals - albite and quartz crystals (Figures 3-1A and 3-1B). The sequestration of U within the dominant minerals makes the U more resistant to leaching and consequently less available for uptake by biota. The moisture contents of the soil and peat were 5.1 % and 18 % respectively.

Table 3- 3: Sequential chemical extraction of U from control and U-treated substrates

	Soil treatment (mg/kg)				Peat treatment (mg/kg)			
Mineral Phase	Control	100	250	400	Control	100	250	400
Exchangeable phase	0.06	8.8	16.5	23	0.48	16.3	37	41
Carbonate phase	3.9	11	20	26.6	0.02	9.02	18	16
Fe-Mn oxide phase	34	38	50.3	67	<0.01	3.9	9	7
Organic phase	9	9	16.2	24.3	5.7	36	59	81
Residual phase	19	26.1	47	78	6	4.8	5	3

The differences between the sum of uranium fractions from the sequential extraction regime and concentrations determined by total digestion of the substrate were remarkable. For instance, while the sum of U fractions in the control soil was 66 mg/kg, the total uranium content determined via aqua regia digestion was 153 mg/kg. This indicates the incomplete liberation of U sorbed on to crystalline mineral and/or organic phases in soil by the extractants.

Results of sequential extraction (Table 3-3) revealed that uranium was largely sequestered onto Fe-Mn oxide and residual phases. The fraction of uranium adsorbed to exchangeable phases was < 0.08 % of the total U determined in soils. On the contrary, the organic phase was the dominant geochemical phase in peat where U interacts with polar organic functional groups through cation exchange and complexation reactions with humic and fulvic acids in peat (Stevenson, 1994). Mechanisms of acidification in peat

have been recognized as: cation exchange, dissociation of organic acids, and sulphate reduction (Clymo, 1964; Stevenson, 1994).

The sequential chemical extraction data also highlighted a general increase in the exchangeable (bioavailable) fraction of U with increasing substrate concentration of uranyl nitrate (Tables 3-3). This could be due to the relatively high acidity of peat. An exhaustion of the cation adsorption and/or exchangeable sites in peat could also account for the relatively high exchangeable fraction. Uranium concentrations in the exchangeable fraction of non-amended peat (control) was determined to be below the detection limit (0.01 ppm); however, concentrations of 1.4 mg/kg, 5.5 mg/kg, and 18.03 mg/kg were respectively determined in peat treated with doses of 100, 250, and 400 mg/kg uranyl nitrate hexahydrate. Uranyl nitrates are generally soluble at acidic pHs and readily dissociate into mobile uranyl (UO_2^{2+}) species available for incorporation into biota (Langmuir, 1978; Ebbs et al., 1998).

It has long been established that the total concentration of a metal in a substrate is a poor indicator of its bioavailability (Hamon and McLaughlin, 2003; Kidd et al., 2007). Uranium exists in several different chemical forms in substrates, and it is important to recognize that the most readily available forms are the soluble forms transported as uranyl ions (UO_2^{2+}) under acidic conditions or hexavalent carbonate complexes (Negri and Hinchman, 2000) at alkaline pH of substrate pore water. An example is shown in Tables 3-3 and 3-4, where substantial differences were reported for total U content and available concentrations in soil and peat. Consequently, plant metal bioavailability is a function of the metal availability index determined as the ratio of available metal concentration

(mg/kg) in a substrate to the total metal content (mg/kg) of a substrate. Metal availability indices computed in this study (Tables 3-4 and 3-5) suggest greater solubility and mobility of uranyl species in peat than in soil.

Table 3- 4: Total U concentration in U-treated soils and bioavailability indices for U after four weeks of stabilization

Treatment (mg/kg)	Mean (mg/kg)	Maximum (mg/kg)	Minimum (mg/kg)	Bioavailable (mg/kg)	Bioavailability index
Control	153	161	115	3.07	0.02
100	288	310	263	10.7	0.04
250	330	427	357	16.5	0.05
400	513	548	501	26	0.05
Determined after sixteen weeks of plant growth					
250/month	449	482	423	14.03	0.03

Table 3- 5: Total U concentration in U-treated peat and bioavailability indices for U after four weeks of stabilization.

Treatment (mg/kg)	Mean (mg/kg)	Maximum (mg/kg)	Minimum (mg/kg)	Bioavailable conc. (mg/kg)	Bioavailability index
Control	6	12	2.4	0	<0.01
100	115	132	111	24	0.21
250	252	263	245	37	0.15
400	422	451	392	41	0.10
Determined after sixteen weeks of plant growth					
250/month	371	407	339	15.1	0.04

Since metal bioavailability is highly correlated with the bioavailability index in a substrate, a higher U uptake was expected in seedlings grown in potting peat dosed with uranyl nitrate solutions. However, U uptake and accumulation in roots and stems of seedlings grown in U-enriched peat were substantially lower than the concentrations determined in seedlings grown in soils treated with uranium. The statistically significant differences ($P < 0.01$) between U content in plants grown in U treated peat and those grown in U-dosed soil suggests that uranium uptake in spruce trees is not entirely a function of metal availability indices. Other abiotic factors may discriminate against root development and metal uptake. Specifically, mechanisms associated with the development of root rhizosphere and mycorrhyzae can be pH sensitive; hence, their development may be inhibited by the acidic pH conditions in peat (Shtangeeva, 2008). In addition, protons and phytosiderophores released by the root are known to greatly influence the microenvironment and significantly impact cation uptake rates of plant roots (Laurie and Manthey, 1994).

3-3-1 VISUAL SYMPTOMS AND PLANT GROWTH

The growth of the seedlings was normal at all doses of substrate U. No visible signs of metal toxicity were observed, even in pots where the total U concentration exceeded 500 mg/kg. A trend of higher biomass production was observed with increasing dose of uranyl nitrate hexahydrate. An increase attributed to concomitant increase in the nitrate content of the substrate as the dose of the U spike (uranyl nitrate hexahydrate) increases. Slight chlorosis was observed on the needles of plants grown in the experimental control

pots (unspiked growth media), but plant growth was not affected. The highest biomass growth was observed in plants grown in pots treated with 400 mg/kg dose followed by those grown on media treated with 250 mg/kg uranyl nitrate hexahydrate.

3-3-2 URANIUM ACCUMULATION IN PLANT PARTS

The content of U and plant nutrients, in particular, Ca, P, and Ni in the black spruce seedlings after 16 weeks growth in the U-dosed growth media (peat and soil) clearly showed preferential accumulation in the stem and followed the order: Ca> U> P> Ni. Maximum U accumulation values of 336 mg/kg, 78 mg/kg, and **7** mg/kg were determined in the stem, roots, and needles, respectively, of plants grown in soils dosed with 400 mg /kg and maximum values of 112 mg/kg, 34 mg/kg, and 2.14 mg/kg were detected in the stems, roots, and needles of seedlings grown in pots containing peat dosed with a 400 mg/kg U (Fig. 3-2A and 3-2B). Similarly, the uptake and mean concentration of Ca, P, and Ni in stems of seedlings increased from 0.5 wt (%), 684 mg/kg, and 0.56 mg/kg respectively to 0.6 wt (%) Ca, 737 mg/kg P, and 0.7 mg/kg Ni in seedlings cultivated in soils treated 400 ppm uranyl nitrate.

The U concentration in the stem of the seedlings also increased monotonically with increasing dose. In particular, the measured U concentration in the stems of spruce seedlings grown in the soils dose with 100, 250, and 400 mg U/kg soil were, respectively, 10, 24, and 50 fold greater than concentrations determined in the stems of control plants. A similar pattern of accumulation was observed in the roots and stems of plants grown in pots containing U-dosed peat. This observation is consistent with the findings of Dunn

(1981, 2007) who reported that U preferentially accumulates in younger twigs of black spruce trees. Analysis of variance indicated significant differences ($p < 0.01$) between the mean U concentrations in the stems and roots of plants grown on U-dosed substrates and those grown in the control group suggesting a greater U availability and uptake in the amended substrates.

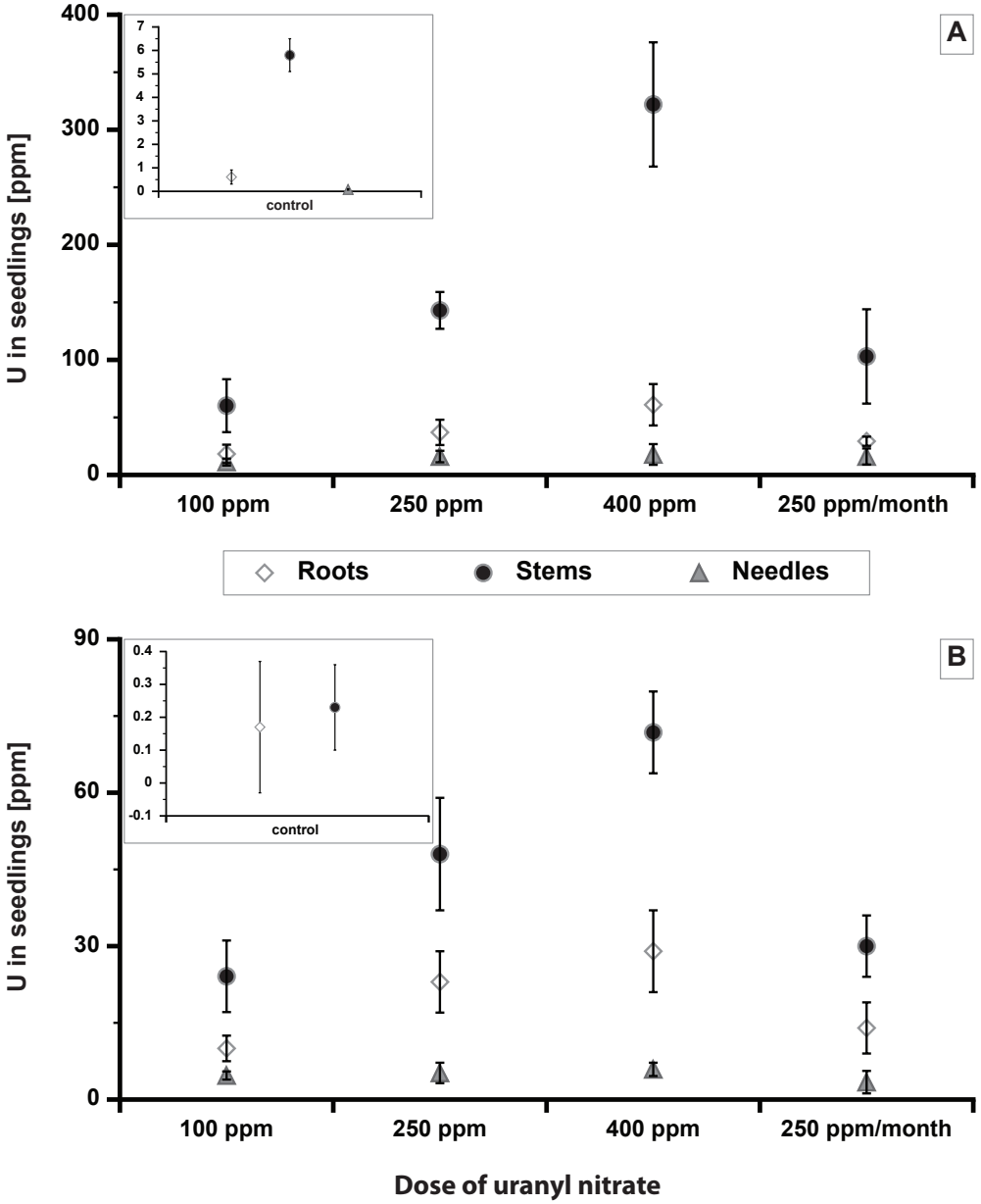


Figure 3- 2: (A) Uranium concentration in roots, stems, and needles of black spruce seedlings after 16 weeks of growth in soil treated with varied doses of uranyl nitrate hexahydrate. (B) Uranium concentration in roots, stems and needles of spruce trees after 16 weeks of growth in peat spiked with varied doses of uranyl nitrate hexahydrate.

As mentioned previously, the results indicated substantial differences in the magnitude of metal accumulation between parts of plants grown on U-spiked peat and those grown in pots containing treated soils. This is consistent with the results of Sheppard and Evendon (1998) and Ramaswani et al. (2001) who observed that U availability and uptake by plants is influenced by substrate heterogeneity. Thus Ramaswami et al. (2001) reported in a comparative study of radionuclide uptake and translocation in plants grown in two different soil types (a sandy-loam soil and a soil rich in organic matter) and a hydroponic medium that the efficiency of U uptake decreased sharply in the order hydroponic > sandy-loam > soil rich in organic matter. The authors concluded that soil organic matter sequestered the U, making it less available for plant uptake. Similarly, Sheppard and Evendon (1998) reported diverse concentrations of U in the stems and leaves of sunflowers grown in several different types of soils contaminated with U. Their study revealed a higher accumulation in the tissues of plants grown in calcareous soils than those grown in acidic soils. The variability of U uptake in response to substrate heterogeneity was largely attributed to substrate specific properties that influence U chemistry.

No significant differences were observed between the uptake of essential plant nutrients in cultivars grown on control media and those grown on U-enriched substrates. However, the treatments of the soils significantly increased the uptake of Fe, Cu, and Al with increasing dose of uranyl nitrate. In particular, mean concentrations of Fe, Cu, and Al in stems of seedlings grown in control soil increased from 310 mg/kg, 16 mg/kg, and 505 mg/kg respectively to 404 mg/kg, 28 mg/kg, and 631 mg/kg in stems of seedlings

grown in soil dosed with 400 ppm uranyl nitrate. The mechanism responsible for increased uptake of Fe, Cu, and Al at a higher dose of uranyl nitrate is unknown, however, Shtangeeva (2008), explained that metal uptake mechanisms in plants are selective and are influenced by plant metabolic activities, consequently plants may acquire some elements over others. Elsewhere, the presence of elevated nutrient levels in substrates was reported to correlate with a higher uptake of U as a consequence of a higher metabolism (Negri and Hinchman, 2000).

Concentrations of U determined in spruce needles were markedly low. Mean concentrations in the young needles grown in control soil were 0.08 mg/kg, and ranged up to 9.8 mg/kg when grown in soil dosed with 400 mg/kg of a uranyl nitrate solution. Generally, concentrations determined in the needles were well within the ranges previously reported in field studies. For example, mean U concentrations of 0.069 mg/kg and 0.37 mg/kg were reported respectively in young and old needles collected from trees located in background areas of a bog habitat located near the Key Lake uranium mill in northern Saskatchewan (Thomas, 2000). Dunn (2007) reported a mean U concentration of 1.64 ppm in matured needles collected in a regional biogeochemical survey at the McClean Lake area of northern Saskatchewan, Canada.

The metal content of the roots, stems, and needles of spruce seedlings grown in pots containing substrates treated with a one-time-dose of 250 mg/kg uranyl nitrate hexahydrate were consistently higher than those grown in pots treated monthly with a 50 mL of 250 mg/kg dose of uranyl nitrate solution (Fig. 3-2A and 3-2B). The low uptake of U from the monthly spiked substrates could be attributed factors including low

accessibility of the U spike by plant root systems and heterogeneity in the distribution of uranyl cations along the soil profile in the pots. Thus, the migration of available forms of the metal was constrained by the level to which water containing dissolved solute (uranyl ions) rises by capillary fringe, and consequently, constrained the availability of mobile species in upper layers of the substrates. Processes, including diffusion, advective flow, and capillary rise, were identified as mechanisms for the vertical migration cations in soil (Aspandiar et al. 2004; Cameron et al. 2004).

Figures 3-2A and 3-2B present the trend of U accumulation in roots, stems and needles of black spruce seedlings relative to the dose of uranyl nitrate added to the substrates. In general, mean U contents in the shoots exhibited linear correlations with bioavailable concentrations (exchangeable fraction) of uranium in the U-dosed soil and peat (Figs. 3-3 and 3-4). The elevated U concentration in the seedlings is not unexpected as the acidic substrate pH promotes the solubility and increase availability of uranyl nitrates (Ebbs et al. 1998; Langmuir, 1998). The toxicity levels of the metal in black spruce trees are uncertain, especially since concentrations in excess of 2000 mg/kg were reported in ash of spruce twigs sampled from areas adjacent to the Rabbit Lake open pit operation in northern Saskatchewan, Canada (Dunn, 2007).

The quantities of metals taken up by plants according to Markert (1989) vary for different plant species and are greatly dependent on the tolerance and ability of plants to adapt high metal concentrations. Based on the metal plant accumulation characteristics defined by Makert (1989) and depicted in Figure 3-3 as excluders, bioindicators, and accumulators, black spruce trees can be classified as bioindicators U contents in the

underlying substrates (Fig 3-4). The magnitude Ni, Cd, Cu, Cr, and Pb or Zn accumulation in the seedlings were below the threshold defined by Brooks et al. (1995) for metal hyperaccumulator plants (Table 3-6).

Table 3- 6: Comparison of metal content in spruce seedlings to the thresholds for definition of hyperaccumulator plant species

Element	Average range in plant tissues (mg/kg dry weight)	Average in stems of spruce seedlings (mg/kg)		Threshold for hyperaccumulator plants (mg/kg dry weight)
		Control	400 ppm dosed soil	
Ni	0.4-4	0.56	0.7	100
Cd	0.03-0.05	0.002-0.004	0.002-0.005	1000
Cu	2-20	<dl	<dl	1000
Cr	0.2-1	0.017-0.036	0.022-0.034	1000
Zn	15-150	12-18	16-34	10000
Pb	0.1-5	0.06-0.21	0.1-0.3	1000

Maestri et al. (2010)

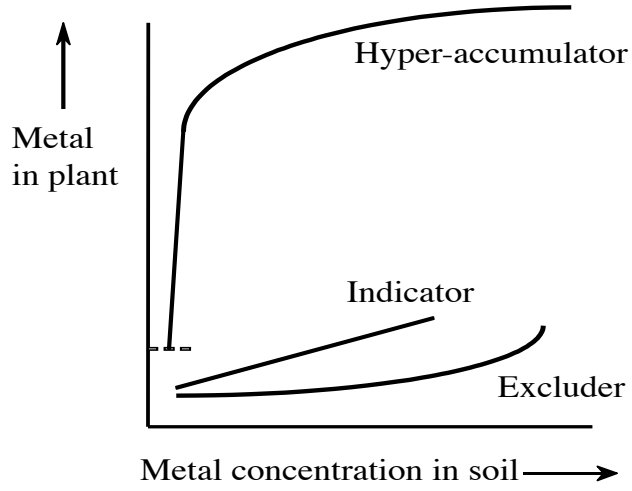


Figure 3- 3: Characteristic relationships between increasing substrate metal concentration and metal accumulation patterns for different categories of plant species (Market, 1989 modified by Hamon and McLaughling, 2003).

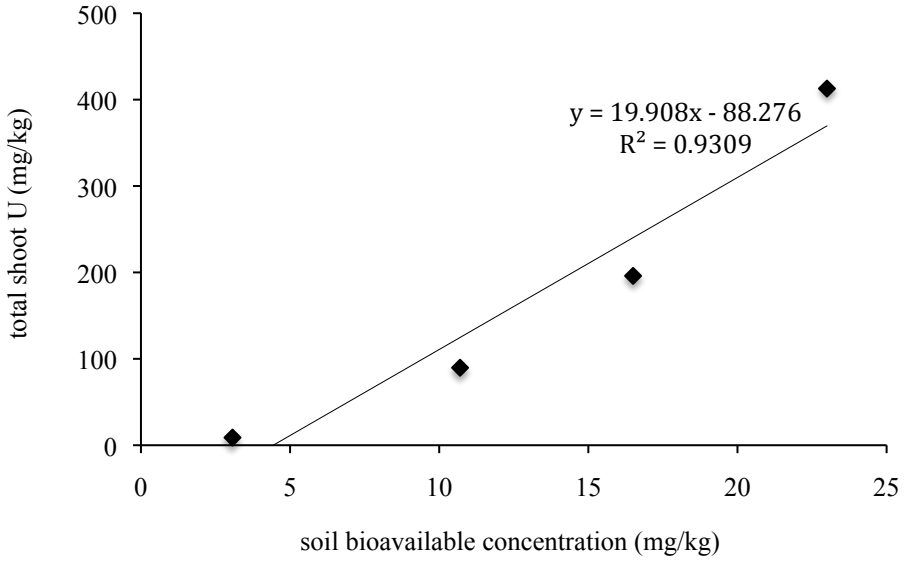


Figure 3- 4: Relationship between bioavailable concentration of U (mg/kg) in soil and total U content in shoots of black spruce seedlings..

The uptake of U from the terrestrial environment by plants is a complicated function of various interrelated biotic and abiotic factors (substrate controls). Factors such as root architecture, rhizosphere chemistry, expression and affinity of root surface transporter proteins for metals, internal compartmentalization of metals and xylem loading of metals, play significantly roles in regulating the speciation of metal cations in the rhizosphere environment (Hamon and McLaughlin 2003, Smical et al., 2008). Plant roots can acidify the rhizosphere by up to 2 pH units compared to the surrounding bulk soil through the release of proton (H^+)/ hydroxide (OH^-), bicarbonates, organic acids and carbon dioxide. (Sylvia et al., 2005; Romheld et al. 1984). Organic chemicals such as mucilage, exudates, and lysates released from the roots into the immediate vicinity of the rhizosphere have the potential of increasing microbiological and biochemical activities which liberate cations from their native adsorption sites in soils. Colonization of the root surface by micorrhizal fungi due to a pH altering effect of rhizosphere increases the absorbing surface area of plant roots and improves the metal uptake from substrate pore water (Sylvia et al., 2005; Kabata-Pendias & Pendias, 2001; Chunilall et al., 2005).

3-3-3 Bioaccumulation and transfer factors

Table 3-5 presents the bioaccumulation coefficients and transfer factors for U in parts of black spruce seedlings grown in pots treated with varied doses of uranyl nitrate and experimental control. The calculated BAC values showed a linear association with the dose of U applied to the substrates (Fig. 3-5) suggesting that the magnitude of the

metal accumulated in the plant tissues is a function of the available concentration to which the plant is exposed. Thus the data displayed in Table 3-5 clearly demonstrate marked differences in the levels of U accumulation in different parts of spruce seedlings. The elevated BAC in the stems confirms the existence of an efficient metal transport and storage system for accumulation of U in the aerial parts of the plant. The metal translocation to the above ground tissues is often associated with transpiration pull. This observation is in contrast with the results by Ramaswami et al. (2001) and Shtangeeva (2008) who reported a higher BAC for U accumulation in the roots of herbs, including, sunflower, Indian mustard, spring vetch, bush bean, hairy vetch, juniper, rye grass, and wheat grown in U contaminated soils, nonetheless, it showed similar trend of increased accumulation with increasing dose in the substrate.

The metal transfer from the stem to needles denoted by $TF_{needles}$ in table 3-5, however, shows marginal translocation of U from the stem to the needles. This implies that there are not specialized mechanisms for storage of U in the needles. It also raises the question of whether the needles lack the mechanisms to compartmentalize metal or the U translocated to the needles gets volatilized into the atmosphere. The data of U accumulation in needles is consistent with the findings of a previous study, where U spruce needles sampled from areas of known uranium mineralisation in the Athabasca region of Saskatchewan returned very low uranium concentrations (Dunn, 1981, 2007). Dunn (1981) suggested that the low enrichment in needles could be explained by barrier mechanisms that potentially inhibit the transport of the metal along the vascular system to the needles. Sheppard and Eventon (1998) and Shahandeh and Hossner (2002) attributed

the low accumulation of U in the aerial parts of some vascular plants to limited mobility of cations along plant vascular systems and cell membrane due to the tendency of cations to adsorb on to cell wall materials.

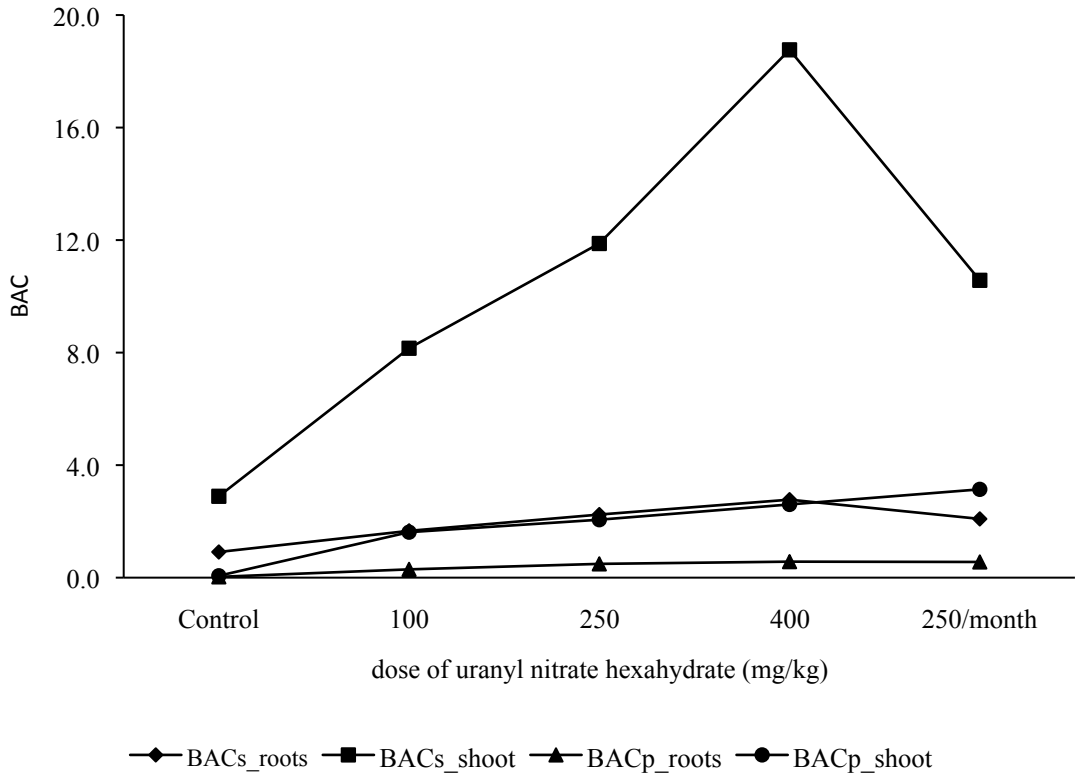


Figure 3- 5: A plot of bioaccumulation factors of U in roots and shoots of black spruce seedlings grown substrates spiked with varied concentrations of U solutions.

The statistically significant differences ($P < 0.01$) between BAF and TF values of plants grown in U-enriched soils and those grown on U-enriched peat (Table 3-5) agree with the observations of Cummins (1994) that bioaccumulation and bioconcentration factors for a metal in a particular species are greatly affected by substrate specific

properties and environmental conditions. The BAC and TF values determined in plants grown on peat treated with 250 mg/kg uranyl nitrate on a monthly basis were significantly greater than those determined in plants grown on peat treated with a single dose of a 250 ppm U solution (Table 3-5).

Table 3- 7: Bioaccumulation and transfer factors for uranium uptake in spruce seedlings grown on substrates treated with varied concentration of U.

Substrate	Soil				Peat			
Treatment (mg/kg)	BAC (roots)	BAC (shoots)	TF (stem)	TF (needles)	BAC (roots)	BAC (shoot)	TF (stem)	TF (needles)
Control	0.91	2.89	2.14	0.01	0.03	0.07	1.35	0.00
100	1.66	8.15	3.29	0.19	0.29	1.62	2.41	0.20
250	2.24	11.88	3.86	0.11	0.49	2.06	2.09	0.11
400	2.77	18.76	5.28	0.09	0.57	2.60	2.48	0.08
250/month	2.09	10.57	3.52	0.16	0.56	3.14	2.14	0.11

In spite of the considerable interest in the evaluation of U uptake and bioaccumulation factors in plants, insufficient data exist relating uptake to the fraction of U that is actually available to plants (Negri and Hinchman, 2000). In other words, U accumulation ratios in most studies were computed using the total U content in the substrates and invariably underestimates the accumulation factors. This is because plants can only access the bioavailable portion of metals present a substrate due to solubility and perhaps spatial constraints. The lack of agreement in the literature on the computation of the metal accumulation ratios in plants and the obvious substrate controls on metal availability create difficulties in comparing metal accumulation ratios from different studies.

3-4 CONCLUSIONS

Although U is not known to be essential or beneficial to plants, many species will absorb and incorporate it into their biomass along with other metals. Results from this study show that black spruce plants are capable of accumulating anomalous quantities of metals in their tissue, which reflects the substrate content; however, the level of accumulation is greatly dependent on the degree of metal availability in the substrate. The magnitude of metal accumulation in parts of black spruce plants followed the order stem > roots > needles. The trend of U accumulation was comparable for plants grown in U-enriched peat and soil, although the magnitude of metal accumulation differed markedly. Uranium accumulation factors (BAF) were higher in shoots compared to values determined in roots. The transfer of the metal to needles was relatively slow in plants grown in soil and peat with TF $\leq$ 0.2. Based on the determined BAF and TF values and the magnitude of accumulation of U, black spruce can best be described as a bioindicator of the metal since the U content in the plant parts examined increased linearly with increasing available concentration in the substrates. Consequently, the magnitude of U uptake is a function of the available metal content in the substrate. It is evident from the experimental data that substrate controls have a significant impact on U uptake by the black spruce trees. More importantly, metal availability in the substrate appears to be the dominant factor responsible for U uptake and accumulation in the organs of black spruce.

3-4-1 IMPLICATIONS FOR MINERAL EXPLORATION AND ENVIRONMENTAL MONITORING

Exploration challenges have changed over the past decade and future discoveries of ore deposits are expected to shift to regions overlain by transported overburden. Exploration geologists consider transported overburden to be an impediment to mineral prospecting; however, evidence now exists that metal accumulating plants growing on exogenous cover can delineate signatures of buried mineralisation. Results from this study indicate that black spruce stems are capable of providing variably focused expression of concealed uranium mineralisation through the uptake and accumulation of U in the stems. The linearity of U accumulated in parts of spruce trees with the available concentrations in substrates provided a great contrast between background and areas of elevated uranium concentration. This property, coupled with the ability of black spruce trees to interact with many cubic metres of soil, groundwater and bedrock through the extensive system, makes black spruce an excellent sampling medium for expressing mineralization haloes in areas overlain by impervious cover material. Compared with mineral prospecting methods such as drilling and soil geochemical surveys, which are arduous to undertake and very expensive, plant sampling and analysis is fast, relatively inexpensive, and has minimal to no environmental impact. As a bioindicator, the black spruce plant has great potential for use as a tool for environmental monitoring as the metal content in the plant tissue provides a direct expression of pollutant impact on the environment that is not readily obtainable by other environmental monitoring techniques. It is apparent from this study that U uptake by spruce trees is substrate-dependent and greatly influenced by the available metal pools in the underlying substrate.

Acknowledgements

Special thanks to the Director of Research, Madonna Bishop, Memorial University Botanical Gardens for providing access and logistical support. The technical support provided by Tim Walsh and Todd Boland, the botanists in charge of the nursery and greenhouse, is very much appreciated. Lakmali Hewa and Pam King of the ICPMS facility at CREAIT, MUN are thanked for their sample preparation and analysis. Bill Clarke is acknowledged for providing black spruce seedlings. Funding was provided in part from the Research Development Council, NL, grant awarded to Garry Thompson. Henry Longerich and Derek Wilton acknowledge support from the Natural Sciences and Engineering Research Council of Canada in the form of Discovery grants.

References

Al-Qahtani K.M. (2012). Assessment of heavy metal accumulation in native plants species from soils contaminated in Riyadh city, Saudi Arabia. Life Science Journal, 9(2): 384-392.

Anand, R.R.; Cornelius, M. and Phang, C. (2007). Use of vegetation and soil in exploration in areas of transported overburden, Yilgarn Craton, Western Australia: a contribution towards understanding metal transportation processes. Geochemistry: Exploration, Environment, Analysis, 7: 267-288.

Aspandiar, M.F.; Anand, R.R.; Gray, D.J. and Gucuzza, J. (2004). Mechanism of metal transfer though transported overburden with the Australian regolith. Explore, 125: 9-12.

Baker, A.J.M. and Brooks, R.R. (1989). Terrestrial higher plants which hyperaccumulate metallic elements – a review of their distribution, ecology and phytochemistry. Biorecovery 1: 81-126

Brooks, R.R. (1983). Biological methods of prospecting for minerals. John Wiley & Sons, NY.

Brooks, R.R.; Dunn, C.E. and Hall, G.E.M. (1995). Biological systems in mineral exploration and processing. Ellis Horwood, Herfortdshire.

Cameron E.M.; Hamilton, S.M.; Leyborne, M.I.; Hall, G.E.M, and McClenaghan, M.B. (2004). Finding deeply buried deposits using geochemistry. Geochemistry: Exploration, Environment, Analysis, 4: 7-432

Cannon, H.L. (1961). Botanical prospecting for ore. Virginia Minerals 7(1): 1-11.

Chunilall, V.; Kindness, A. and Jonnalagadda, S.B. (2005). Heavy metal uptake by two edible *Amaranthus* herbs grown on soils contaminated with Lead, Mercury, Cadmium and Nickel. Journal of Environmental Science and Health, 40: 375-384.

Clymo, R.S. (1964). The origin of acidity in *Sphagnum* bogs. The Bryologist 67: 427-431.

Cummins, C.L. (1994). Radiological bioconcentration factors for aquatic, terrestrial, and wetland ecosystems at Savannah River Site. Westinghouse Savannah Company. Aiken, South Carolina.

Dunn, C.E. (1981). The biogeochemical expression of deeply buried uranium mineralization in Saskatchewan, Canada. Journal of Geochemical Exploration, 15: 437-452.

Dunn, C.E., (2007). Biogeochemistry in mineral exploration. Vol. 2, Elsevier, Amsterdam.

Ebbs, S.D.; Brady, D.J. and Kochian, L.V. 1998. Role of uranium speciation in the uptake and translocation of uranium by plants. Journal of Experimental Botany 49(324): 1183-1190.

Gordon, S. (1992). Link between ore bodies and biosphere concentration of uranium, AERCB Project #5.140.1. Atomic Energy Control Board, Ottawa, Canada.

Hamon, R. and McLaughlin, M. (2003). Food crop edibility on the OK Tedi/Fly river flood plain, Australian Center for Environmental Contaminants Research.

Kabata-Pendias, A. and Pendias, H. (2001). Trace elements in soils and plants. 3rd Ed., CRC Press LLC, Boca Raton.

Kidd, P.S.; Dominguez-rodriguez, M.J.; Diez, C. and Monterroso, J. (2007). Bioavailability and plant accumulation of heavy metals and phosphorus in agricultural soils amended by long-term application of sewage sludge. Chemosphere, 66: 1458-1467.

Kramer, U. (2010). Metal hyperaccumulation. Annual Reviews of Plant Bioliogy, 61: 517-534.

Langmuir, D. (1978) Uranium solution-mineral equilibria at low temperatures with applications to sedimentary ore deposits. Geochimica. et Cosmochimica Acta 42: 547-69.

Laurie, S. H. and Manthey, J. A. (1994) The chemistry and role of metal ion chelation in plant uptake processes. In: Biochemistry of Metal Micro- nutrients in the Rhizosphere. J. A. Manthey, D. E. Crowley, D. G. Luster (Eds.). CRC Press, Boca Raton, FL, pp: 165-182.

Maestri, E., Marmiroli, M., Visioli. G., Marmiroli, N. (2010). Metal tolerance and hyperaccumultion: Cost and trade-offs between traits and environment. Environmental and Experimental Biology, 68: 1-13

Markert, B. (1993) Plant as biomonitors / indicators for heavy metals in the terrestrial environment. VCH Press, Weinheim. In: Monaci F. and R. Bargali, (2000). Biomonitoring of airborne metals in urban environments: New trace of vehicle emission in place of lead. Environmental Pollution, 107: 321- 327.

Negri, C.M. and Hinchman, R.R. (2000). The use of plants for the treatment of radionuclides. In: Phytoremediation of toxic metals using plants to clean up the environment. Raskin I., B.D. Ensley (Eds), John Wiley & Sons, Inc., Toronto, pp: 118-123.

Ozturk, M.; Yucel, E.; Gucel, S.; Sakcali, S., and Aksoy, A. (2008). Plants as biomonitors of trace elements pollution in soil. In: Trace elements as contaminants and nutrients. Prasad M.N.V. (Ed), John Wiley & Sons Inc., Hoboken, NJ, pp: 721-735.

Ramaswami, A.; Carr, P. and Burkhardt, M. (2001). Plant-uptake of uranium: hydroponic and soil system studies. International Journal of Phytoremediation 3(2): 189-2011.

Reid, N. and Hill, S.M. (2010). Biogeochemical sampling for mineral exploration in arid terrains: Tanami Gold Province, Australia. Journal of Geochemical Exploration, 104: 105-117.

Romheld, V.; Muller, C. and Marschner, H. (1984). Localization and capacity of proton pumps in roots of intact sunflower plants. Plant Physiology, 76**:** 603-606.

Selinus, O. (1988). Biogeochemical mapping of Sweden for geomedical and environmental research, In: Thornton I. (Ed). Proceedings of the 2nd Symposium on geochemistry and health. Northwood: Science Reviews Ltd.

Selinus, O. (1989). Heavy metals and health-results of the biogeochemical mapping program of Sweden. EUG V. Strasbourg.

Shahandeh, H. and Hossner L.R. (2002). Role of soil properties in phytoaccumulation of uranium. Water, Air, and Soil Pollution, 141: 165-180.

Sheppard, S.C. and Evendon, W.G. (1988) Critical compilation and review of plant/soil concentration ratios of uranium, thorium, and lead. Journal of Environmental Radioactivity, 8: 255-258

Shtangeeva, I. (2008). Uranium and thorium accumulation in cultivated plants. In: Trace elements as contaminants and nutrients. Prasad M.N.V. (Ed), John Wiley & Sons Inc., Hoboken, NJ, pp: 721-735.

Smical, A.; Hotea, V.; Oros, V.; Juhsz J.; and Pop, E. (2008). Studies on transfer and bioaccumulation of heavy metals from soil into lettuce. Environmental Engineering and Management Journal, 7(5) 609-615.

Stevenson, F.J., (1994). Humus chemistry: genesis, composition, reactions. 2nd Edn., John Wiley & Sons. Inc, New York.

Sylvia, D.M.; Hartel, P.G.; Fuhrmann, J.J. and Zuberer, D.A. (2005). Principles and applications of soil microbiology, (2nd Ed). Pearson Prentice Hall, New Jersey, pp: 254-272.

Tessier, A.; Campbell, P.G.C.; and Bisson, M. (1979). Sequential extraction procedure for the speciation of particulate trace metals. Analytical Chemistry, 51: 844-851.

Thomas, P. (2000) Radionuclides in the terrestrial ecosystem near a Canadian uranium mill - Part II: Small mammal food chains and bioavailability. Health Physics 78(6): 625-632.

Wilton, D.H.C. (2012). Report on the MLA-SEM analysis of a soil sample from the Mitchelin trench, Central Mineral Belt, Labrador. Aurora Energy Ltd., St John's.

Wilton, H.C. and Winter, L.S. (2012). SEM-MLA research on indicator minerals in Till and stream sediments – an example from exploration for awaruite in Newfoundland and Labrador, In: Quantitative mineralogy and microanalysis. Sylverster P. (Ed), Mineralogical Association of Canada short course 42, St John's, NL, pp: 265-284.

Appendix 3- 1: Sequential extraction protocol (modified from Tessier et al., 1979)

Exchangeable phase

An aliquot of 0.5 g of the sub-250 μg/g fraction of soil was weighed into 100 mL centrifuge tube and 8 mL of magnesium chloride added, and the pH of the solution adjusted to 7.0. The mixture was centrifuged at 5000 rpm for 30 minutes and the supernatant decanted into acid cleaned TEFLON containers. The solution was evaporated to dryness and the residue dissolved in 2 mL 8 M HNO_3. The resultant solution was diluted gravimetrically to 30 g with deionized water. The soil residue was rinsed with 8 mL deionized water and the rinse discarded.

Carbonate phase

The soil residue from the exchangeable phase was mixed with 8 mL of 1 M sodium acetate and the pH adjusted to 5.0 with acetic acid. The mixture was shaken on a mechanical shaker for 5 hours and then centrifuged at 5000 rpm for 30 minutes. The supernatant was decanted into clean Teflon beakers and then evaporated to dryness. The residue was reconstituted in 2 mL 8 M HNO_3 and gravimetrically diluted to 30 g with deionized water for ICP-MS analysis. The soil residue was rinsed with 8 mL deionized water and the rinse discarded.

Fe-Mn oxide phase

Approximately 15 mL of 0.04 M hydroxylamine hydrochloride in 25% (v/v) acetic acid was added to the soil residue and warmed to 96 ± 5 ^{o}C for 6 hours with occasional agitation. The mixture was centrifuged for 5000 rpm for 30 minutes, the supernatant decanted and residue washed with 8 mL of deionized water. The separated supernatant was evaporated to dryness and residue dissolved in 2 mL of 8 M HNO_3 acid. The resultant solution was gravimetrically diluted to 30 g with deionized for ICP-MS analysis.

Organic matter phase

To the soil residue from the previous step was added 3 mL of 0.02 M HNO_3 and 5 mL of 30% H_2O_2 and the pH adjusted to 2 with drops of HNO_3. The mixture was warmed in a water bath with the temperature set at 85±2 ^{o}C for 2 hours. Upon cooling, 5 mL of 3.2 M ammonium acetate in 20% (v/v) HNO_3 was added and the mixture made up to 20 mL with deionized water. The resultant mixture was centrifuged at 5000 rpm for 30 minutes, the supernatant was decanted into clean Teflon beakers and then evaporated to dryness. The residue, dissolved in 2 mL of 8M HNO_3 was gravimetrically diluted to 30g for ICP-MS analysis. The soil residue was rinsed with 8 mL of deionized water.

Residual phase

The rinsed soil residue was transferred into a Teflon jar and 3 mL of 8M HNO_3/29M HF (2:1) mixture added. The Teflon jar was capped and the mixture heated at

100±2 ^{o}C until the solids were completely dissolved. The resulting clear solutions were evaporated to dryness, the residue reconstituted in 2 mL 8 M HNO_3 and then diluted to 30g with deionized water for ICP-MS analysis.

Total soil digestion

To approximately 0.1g of <250 μm size fraction of soil was added 1 mL each of 8M HNO_3 and 29 M HF in a Teflon jar. The mixture was capped and heated at 90±3 ^{o}C for 24 hours following which it was evaporated to dryness. A 4 mL solution of aqua regia (1mL 8 M HNO_3 and 3 mL of concentration HCl) was added to the residue and heated on a hot plate at 90±3 ^{o}C for 48 hours and then evaporated to dryness. Another 4 mL of aqua regia solution was added to resultant residue and again heated on a hot plate for 24 hours. This step was repeated until all the solids dissolved and a clear solution obtained. The clear solution was evaporated to dryness, the resultant residue reconstituted in 2 ml of 8M HNO_3, and then gravimetrically diluted to 60 g for ICP-MS analysis.

References

Tessier, A.; Campbell, P.G.C., and Bisson, M. (1979). Sequential extraction procedure for the speciation of particulate trace metals. Analytical Chemistry, 51: 844 – 851.

Appendix 3- 2: Element concentration in standard reference materials and Michelin soil

Element	Unit	NIST SRM 2710a		NIST SRM 2711a		Michelin soil
		Measured	Certified	Measured	Certified	
Li	ppm	20.8	ND	19.6	ND	5.9
B	ppm	59466	ND	54170	ND	35646.1
Mg	ppm	4900.25	10500	6783.0	ND	1367
Al	ppm	24714.5	65300	26768	65300	26033.3
Si	ppm	123239.0	304400	141905	304400	147120
P	ppm	831.5	860	642.3	860	1631
S	ppm	<0.001	ND	<0.001	420	0.12
Ca	ppm	2084.5	9640	14304.4	28800	8411
Ti	ppm	2624	3060	2666.8	3060	1920
V	ppm	63.05	81.6	63	81.6	40.6
Cr	ppm	23	23	44.3	47	11
Mn	ppm	1834.2	638	570.98	638	520.6
Fe	ppm	40341.3	43200	28056.3	28900	18757
Co	ppm	6.4	10	9.8	10	3.6
Ni	ppm	12.4	20	25.7	20.6	9.6
Cu	ppm	2926.7	114	131.7	114	<0.02
Zn	ppm	3905.7	4180	389.8	350.4	56.2
As	ppm	1155.07	105	34.8	105	1.9
Rb	ppm	110.0	110	116	110	24
Sr	ppm	243.45	245.3	224.66	245.3	74.6
Mo	ppm	7.6		1.6	1.6	1.7
Ag	ppm	48.8	40	7.9	4.63	1.3
Cd	ppm	11.3	12	49.7	41.7	0.3
Sn	ppm	12	4.1	8	4.1	7.3
Sb	ppm	48.3	52	23	19.4	0.4
Cs	ppm	7.49	8.25	6.19	6.1	0.43
Ba	ppm	711.9	726	650.2	726	329.7
La	ppm	27.6	30.6	32.61	40	20.05
Ce	ppm	53.6	60	65.8	69	48.2
Hg	ppm	4.2	6.1	ND	ND	<0.01
Tl	ppm	1.3	1.6	2.44	2.47	0.1
Pb	ppm	3794.9	1111.7	1088	1162	19
Bi	ppm	45.6	23	3.12	22.3	0.4
U	ppm	8.8	9.1	2.7	3.01	153

ND = not detected

Appendix 3- 3: Peters Professional started fertilizer 10-52-10

The plant starter fertilizer with major nutrient composition of 10% N, 52% P, and 10% K was applied to substrates prior to seedling transplant. The fertilizer contains the phosphorus necessary (52%) for rapid root development and growth. The presence of adequate N helps in leaf (needle) development whereas potassium is responsible for root development and the plant's defense against to diseases. This fertilizer was applied once a week for the first two-weeks after transplanting. Aliquots of 50 mL of 2 g/L fertilizer solution were added to each pot containing 500 g of substrate (i.e. soil or peat).

Nutrient	Percentage (%)
Total nitrogen	10
Ammoniacal nitrogen (NH_4-H)	7.6
Urea nitrogen (Ur-N)	2.4
Phosphorus pentoxide (P_2O_5)	52
Soluble phosphorus (P)	22.6
Potassium oxide (K_2O)	10
Micronutrients	
Boron	0.02
Copper chelated by EDTA	0.015
Iron chelated by DTPA	0.12
Manganese chelated by EDTA	0.06
Molybdenum	0.01
Zinc	0.015

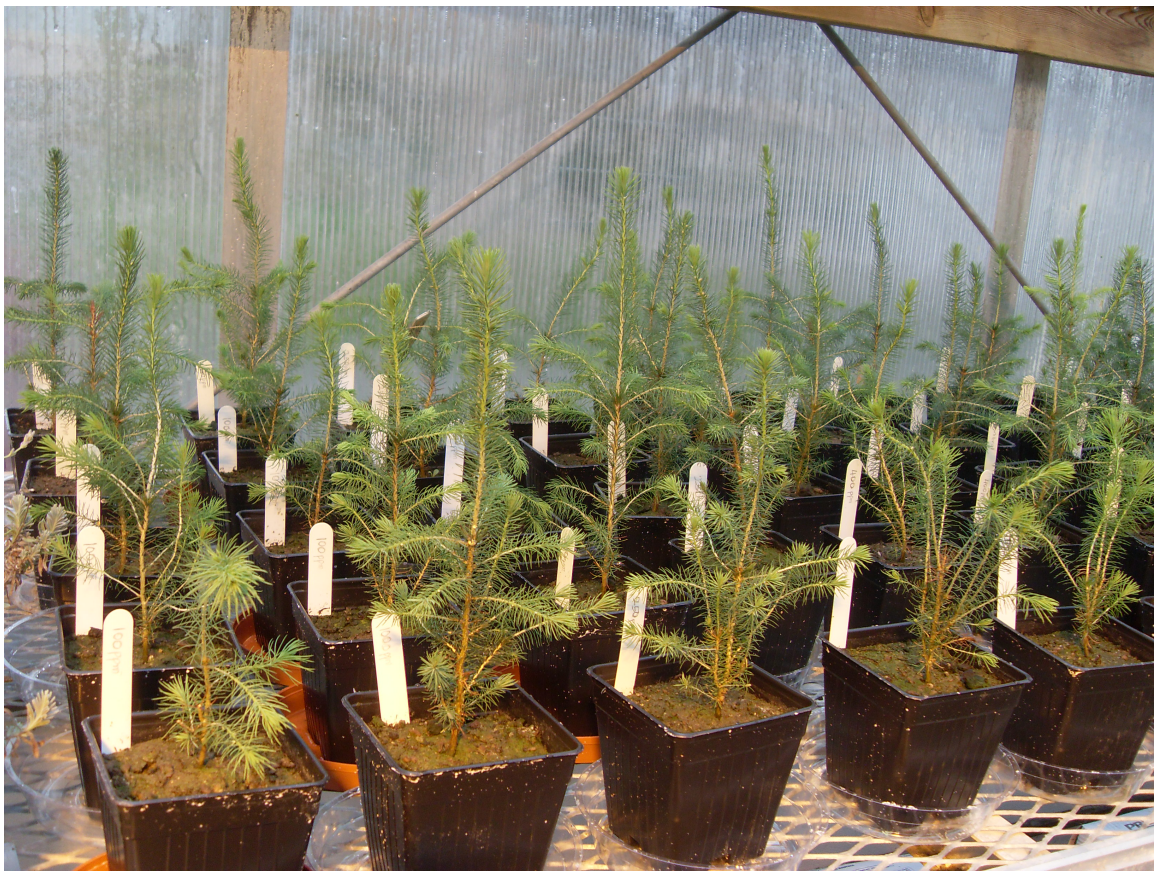
Appendix 3- 4: Peters Professional general-purpose fertilizer 20-20-20

The general purpose fertilizer contains equal amounts of each major nutrient (i.e. 20 % by weight of N, P, K) and is intended to meet the plants' general nutrient requirements once the roots were fully developed. The fertilizer contains inorganic nutrients that are in forms most easily absorbed by plants. The major and micronutrient composition of the 20-20-20 general-purpose fertilizer is shown in appendix 3-3A

Nutrient	Percentage (%)
Total nitrogen (N)	20
Ammoniacal nitrogen	3.94
Nitrate nitrogen	6.05
Urea nitrogen	10.01
Available phosphate (P_2O_5)	20
Soluble potash (K_2O)	20
Minor nutrients	
Magnesium (Mg) Total (water soluble)	0.05
Boron (B)	0.0068
Chelated copper (Cu)	0.0036
Chelated iron (Fe)	0.05
Chelated manganese (Mn)	0.025
Molybdenum (Mo)	0.0009
Chelated zinc (Zn)	0.0025

Appendix 3- 5: Black spruce seedlings grown in pots containing peat and dosed monthly with 100 mL of 250 ppm solution of uranyl nitrate

Appendix 3- 6: Black spruce seedlings grown in a pots containing <4mm soil fraction and treated separately with varied concentration of uranyl nitrate



Appendix 3- 7: Black spruce seedlings after 16 weeks of growth in substrates treated with 250 ppm uranyl nitrate solution

Appendix 3- 8: Mineral Liberation Analyzer-Scanning Electron Microprobe analysis of the <250 μm fraction of soil used in the greenhouse study

To determine the mineral phases to which uranium was sequestered, the sub 250 μm size fraction of soil used for the greenhouse study was analyzed in the Micro-analytical laboratory of the Bruneau Innovation Centre at MUN using the MLA-SEM protocol developed by Wilton and Winter (2012). The SEM portion of the facility consists of a FEI Qunta 400 environmental SEM equipped with Bruker ZFlash EDX Detector. The MLA software associated with the SEM allows for quantitative evaluation of the abundance, associations, size and shape of mineral in an automated, systematic procedure (Wilton and Winter, 2012). The MLA software use the SEM in the Back Scatter Electron (BSE) image mode in which minerals having a higher mean atomic number of the elements produce brighter images. The software detects mineral particles in the mount based on variation in the BSE grey scale and then analyses each particle. The MLA maps each grain mount in terms of frames per mount, typically about 100 frames; each frame represents a portion of the mount. The particles in each frame are analysed and then their relative and absolute abundances are calculated.

Prior to the MLA-SEM analysis, 0.3 g aliquot of the <250 μm size fraction of soil was mounted in epoxy, and the mount was polished. The grain mount was polished using a Struers polishing system consisting of Tegra Pol 31 polisher and a Tegra Focus 5 holder, which produced a final polish of 1 μm. The grey-scale "thresholds" were set with the epoxy as black and metallic copper as white. Image resolution was 800 dpi and the

minimum particle detected was about 5 μm in diameter. The MLA mapped 97 frames from the Michelin soil grain mount (Wilton, 2012). Appendix 3-5 lists the mineral phases identified by the MLA-SEM. A total of 17,711 particles were identified in the grain mount.

References

Wilton, D.H.C. (2012). Report on the MLA-SEM analysis of a soil sample from the Mitchelin trench, Central Mineral Belt, Labrador. Aurora Energy Ltd., St John's.

Wilton, D.H.C and Winter, L.S. (2012). SEM-MLA research on indicator minerals in Till and stream sediments – an example from exploration for awaruite in Newfoundland and Labrador, In: Quantitative mineralogy and microanalysis. Sylverster P. (Ed), Mineralogical Association of Canada short course 42, St John's, NL, pp: 265-284.

Appendix 3- 9: Mineral phases identified by MLA-SEM analysis of the soil grain mount from <250 μm sized-fraction of soil

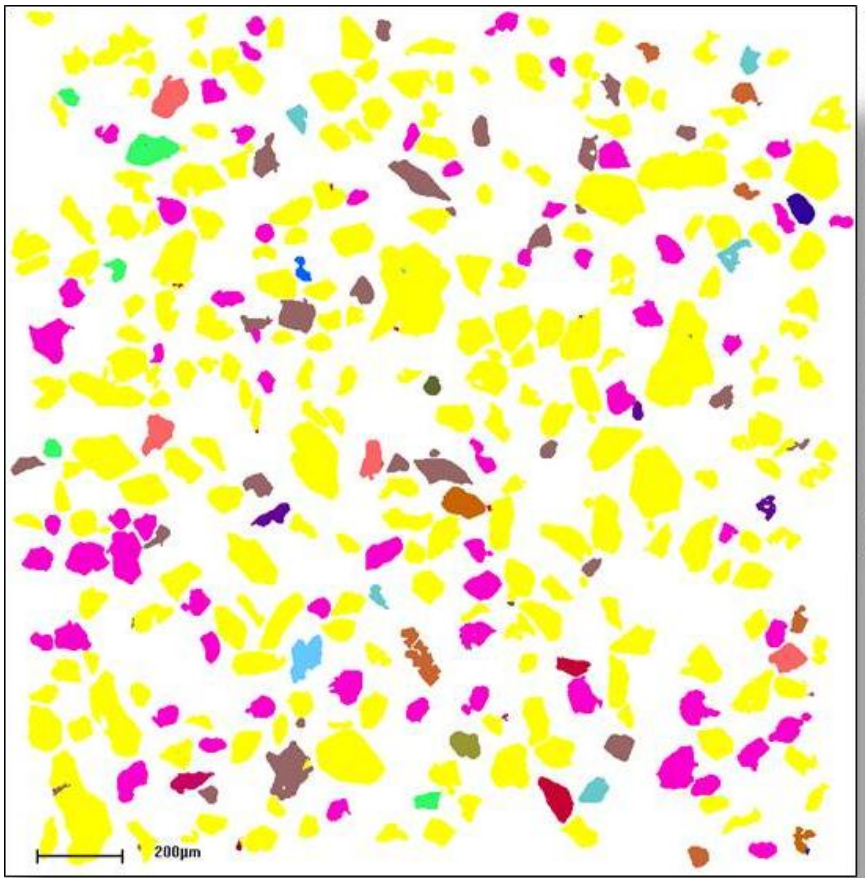
Mineral	Mineral phases (%)
Albite	68
Quartz	17
Low-Fe muscovite	6
*Invalid	2
Hornblend-Fe	1
Plagioclass-An 25	0.89
Perthite	0.68
Ferrorichterite	0.44
Hornblende	0.4
Hypersthene W Ca	0.28
**Unknown	0.27
Grossular	0.26
Orthoclase	0.23
Magnetite	0.19
Titanite	0.12
Biotite	0.1
Zircon	0.1
Si-uraninite	0.01
Pb-uraninite	0.0001

**Unidentified particles are classified as unknown

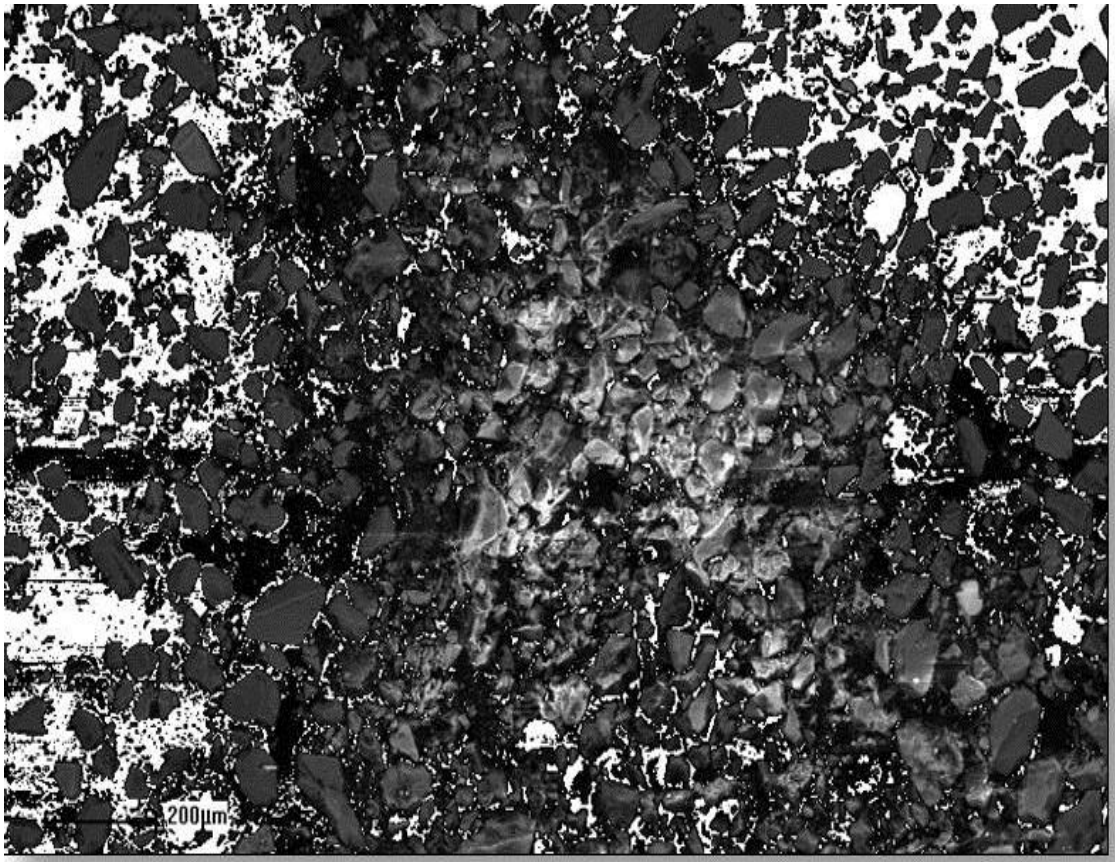
*Invalid particles include areas of electron charging, edges of the grain mount, holes in the mount, etc.

Appendix 3- 10: MLA-SEM false colour. Minerals in the MLA image are albite (yellow) quartz (pink), low-Fe muscovite (olive-brown), hornblende (light blue), ferro- richterite (green), clinopyroxene (dark blue), perthite (red-brown), and grossular (olive).

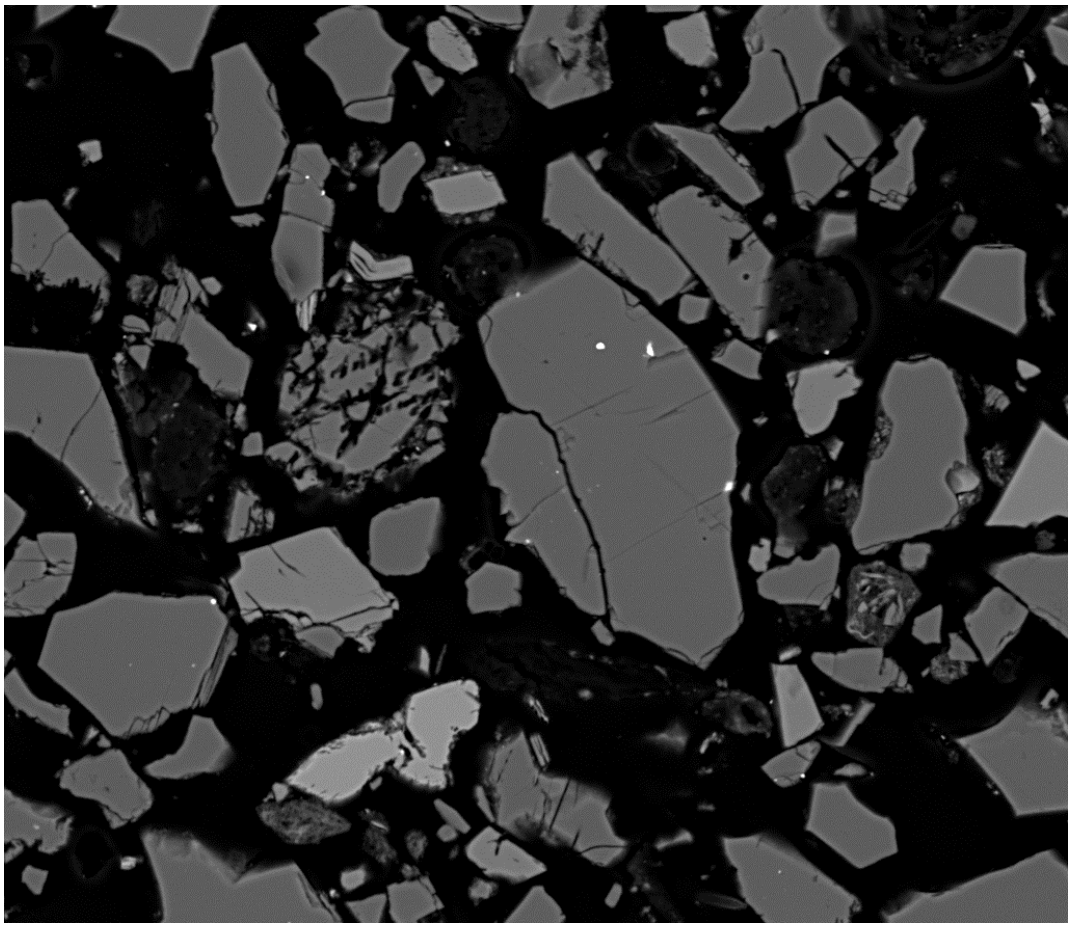
* – The scale bar is 200 µm (Wilton, 2012).



Appendix 3- 11: Back Scattering Electron-Scanning Electron Microprobe image of electron charging (whitish patch, middle view) due to incomplete graphite coating of the sub-250 μm soil grain mount (Wilton, 2012).



Appendix 3- 12: BSE-SEM of 3rd largest Si-uraninite particles (white) in albite, sub-250 μm size fraction of soil grain mount (Wilton, 2012).



Appendix 3- 13: Mean U concentration in parts of black spruce seedlings grown in soils treated with uranyl nitrate.

Soil treatments	Roots	Stems	Needles
Control (0 ppm)	2.8	6	0.08
100 ppm	18.3	60.2	11.2
250 ppm	37	143	16
400 ppm	61	322	29.8
250/month	29.3	103	16

Appendix 3- 14: Mean U concentration in parts of black spruce seedlings grown in peat treated with uranyl nitrate

Peat treatments	Roots	Stems	Needles
Control (0 ppm)	0.17	0.23	<0.001
100 ppm	10	24.1	4.7
250 ppm	23	48	5.2
400 ppm	29	71.8	5.9
250/month	14	30	3.4

Chapter 4

In

Praise K. Nyade, Derek H.C. Wilton, Gary M. Thompson, Henry P. Longerich (2013). Measurement of $^{238}U/^{235}U$ ratio in black spruce seedlings to fingerprint the true source of uranium taken up by metal accumulating plants. Submitted to the Journal of Radioanalytical and Nuclear Chemistry.

Measurement of $^{238}U/^{235}U$ ratio in black spruce seedlings to fingerprint the source of uranium taken up by metal accumulating plants.

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Abstract

This study explored the measurement of $^{238}U/^{235}U$ ratio in black spruce to investigate the true source of U up taken by the seedlings grown in pots treated with depleted uranium (DU). The measurement of the $^{238}U/^{235}U$ ratio in vegetation also has an added advantage of fingerprinting the incidence of anthropogenically-derived uranium in groundwater and in environmental and geological media. Inductively coupled plasma quadrupole mass spectrometer (ICP-QMS) analyses of the $^{238}U/^{235}U$ ratio in stems of spruce seedlings grown in pots filled with 500g substrates (soil, peat) and dosed biweekly with 100 mL aliquots of 100, 250, and 400 mg/L DU produced ratios of 174, 187, and 210 respectively. The $^{238}U/^{235}U$ ratio in the stems increased linearly with increasing available U concentration in the DU-treated substrates. Similarly, the $^{238}U/^{235}U$ measurements in the total acid (HF/HNO_3-aqua regia) digested DU-treated substrates

exhibited a marked deviation from the accepted natural value of 137.88. The increased $^{238}U/^{235}U$ ratio in the seedlings effectively demonstrates that metals absorbed by the plants were derived predominantly from the labile components in water rather than the cations adsorbed on to mineral phases in the substrate. The precision and accuracy of the measured ratios were compared with the $^{238}U/^{235}U$ in the in-house granite and uraninite reference samples. Although both techniques successfully demonstrated the occurrence of isotope disequilibrium in the DU-treated substrates and spruce seedlings, isotope measurements by NAA were characterized by high uncertainty, which while statistically agreeing with the ICP-QMS values were not useful for the study.

4-1 INTRODUCTION

The analysis of trace elements in vegetation has gained acceptance as a reliable exploration tool for outlining buried mineralization, including areas overlain by extensive glacial drift (e.g. Cannon, 1961; Dunn, 1981, 2007; Nyade et al., 2013b). Indeed, the determination of uranium contents in the ash of black spruce twigs and bark defines surface geochemical signatures of buried U ore in the Athabasca Basin of northern Saskatchewan, Canada (Dunn, 1981, 2007). Similarly, the use of plants as biomonitors of environmental pollution has been studied by a number of authors (Markert et al., 1999; Di Guardo et al., 2003; Margui et al., 2005). Various plants have been observed to accumulate anthropogenic pollutants; in particular, heavy metal accumulations were reported in holm oak (Alfani et al., 1996), dandelion (Normandin et al., 1999; Rogstade et al., 2002), and moss (Markert et al., 1999). Conifer needles are useful as biomonitors of atmospheric contaminations of semi-volatile organic compounds, e.g. DDTs, PCBs, PAHs, and Dioxines (Treolada et al., 1994; Di Guardo et al., 1999; 2003).

However, while vegetation analysis has been effective in outlining surface signatures of buried U mineralization and/or environmental toxicants, there is a lack of understanding of true source and pathway of the metals taken up by the plants. That is, whether the metals taken up by vegetation were derived directly from the bedrock and/ or surface soils, or were groundwater constituents absorbed by the plants. Another significant challenge of biogeochemical prospecting for buried U ore is the ability to discriminate between surface geochemical anomalies derived from natural mineralization and the signatures derived from anthropogenic radionuclide contaminations.

Measurements of U isotope ratios may provide useful information for discerning the sources and pathways of U taken up by vegetation. More importantly, the analysis of $^{238}U/^{235}U$ ratio in vegetation serves as a unique technique for discriminating uranium of anthropogenic origin since the $^{238}U/^{235}U$ ratio is essentially invariant in natural U with the single known exception of the ancient natural reactor at Oklo in Gabon (Boulyga and Becker 2002; Chenery et al., 2002). Anthropogenically-derived U is either enriched or depleted in the ^{235}U isotope, consequently the $^{238}U/^{235}U$ ratios of anthropogenically-derived U are substantially different from the accepted natural ratio of 137.88 (Chen and Wasserbug, 1981; Abdelouas, 2006). Table 4-1 summarizes the relative abundances of the naturally occurring isotopes of U in natural samples as well as their typical abundances in enriched and depleted U samples.

Table 4- 1: Isotopic composition of uranium in selected uranium-containing materials

Isotope	Half-life (years)	Relative mass (%)			
		Natural	Enriched	Depleted U	Weapons grade
^{234}U	2.44×10^{5}	0.0058	0.00948-1.0	0.00-0.2	<0.005
^{235}U	7.0×10^{8}	0.72	0.9-93	0.2-0.4	98
^{238}U	4.47×10^{9}	99.27	79.26-98.36	99.6-99.8	2
$^{238}U/^{235}U$		137.77	<3.96-108	249.5-499	2.04×10^{-3}

The goal of this study was to evaluate using the $^{238}U/^{235}U$ ratio in black spruce seedlings grown in pots dosed with aliquots of DU to establish the true source of U taken up by the biogeochemical sampling media. The idea is to discriminate whether the U taken up by the plants is pedogenic or was dissolved in groundwater and then taken up by the plants. A second objective was to examine the applicability of the major U isotope ratio ($^{238}U/^{235}U$) in black spruce seedlings as tracers of the incidence of non-natural U contamination in environmental and geological media. The study utilized cost effective, yet efficient, techniques, including quadrupole-based inductively coupled plasma mass spectrometry (ICP-QMS) and neutron activation analysis (NAA) as "a fit for purpose" procedure to measure the $^{238}U/^{235}U$ ratio in the substrates and spruce seedlings grown on the DU-treated substrates. Although there are several naturally occurring isotopes of U, the $^{238}U/^{235}U$ ratio is best suited for analytical purposes because these two isotopes have the greatest abundance and can thus be measured more precisely and accurately.

4-2 MATERIALS AND METHODS

4-2-1 REAGENTS AND MATERIALS

The acids used for sample preparation were of analytical grade and included: nitric acid (70 % v/v), hydrofluoric acid (40 % v/v), and hydrochloric acid (38 % v/v), and certified A.C.S. ammonium acetate; all supplied by Fisher Scientific (Fair Lawn, New Jersey, USA). Depleted uranium (Uranyl nitrate hexahydrate) and Peters Professional 5-11-26 Hydro-sol water soluble fertilizer were respectively supplied by

BDH Chemicals Ltd (Poole, England) and The Scotts Company (Marysville, Ohio, USA). Solutions of in-house reference materials - a fresh granite rock from the Voisey Bay area, Labrador, and uraninite ore from the Beaver Lodge uranium deposit, Saskatchewan were prepared by HNO_3/HF (1:1)-aqua regia digestion. The choice of a granite rock and uraninite as in-house reference materials is based on the assumption of the accepted natural $^{238}U/^{235}U$ ratio (137.88) in these natural samples.

De-ionized water (nano-pure water, 18 mΩ cm @ 20 ^{o}C) was produced from a Millipore Elix-5/Mill-Q water purification system (Bedford, ON, Canada). The plant nursery of the Forestry Division, Department of Natural Resources, Province of Newfoundland and Labrador, Canada supplied two-year-old black spruce seedlings. Pro-mix potting peat was procured from Premier Horticulture Limited (Richer, MB, Canada) and the bulk podzol soil for the greenhouse pot experiments was obtained from the "discovery" trench at the Michelin U Deposit, Central Mineral Belt (CMB), Labrador, Canada.

4-2-1 INSTRUMENT

A Perkin Elmer Sciex ELAN DRC II, inductively coupled plasma quadrupole mass spectrometry (ICP-QMS) (Concord, ON, Canada) with a Seaspray U-series nebulizer fitted into a standard cyclonic spray chamber and a Simulscan dual-stage detector was used for isotope ratio measurements at the CREAIT facility of Memorial University of Newfoundland. Compared with more conventional nebulizers, the Seaspray U-series nebulizer has the advantage of reducing salt deposition on the orifice of the

nebulizer. Table 4-2 summarizes the instrument settings and data acquisition parameters for the ICP-QMS. Subsamples of the DU-dosed substrates were analyzed for ^{235}U and ^{238}U using Neutron Activation Analysis (NAA) at Becquerel Laboratories Inc. (Mississauga, ON, Canada).

Table 4- 2: Instrument parameters and optimized conditions for the Perkin Elmer Sciex ELAN DRC II ICP-QMS

Instrument settings	
RF power	1250 W
Plasma gas flow rate	15 L/min
Auxiliary gas flow rate	1 L/min
Nebulizer gas flow rate	1.2 L/min
Nebulizer	Babington type (Seaspray U-series)
Spray chamber	Cyclonic
Sample cone	Nickel 1.1 mm
Skimmer cone	Nickel 0.9 mm
Data acquisition settings	
Detector mode	Dual - pulse and analog
Scan mode	Peak hop transient
Lens scanning	Enabled
Resolution	0.7 amu at 10% peak maximum
Isotope measurements	
Isotopes measured	^{235}U, ^{238}U, ^{204}Tl, ^{205}Tl
Sweeps per readings	1
Readings per replicates	300
Number of replicates	1
Dwell time	30 ms

4-2-2 PLANT CULTIVATION IN A GREENHOUSE

Three sets of pots filled with 500 g of oven-dried <4 mm-sized fraction of bulk podzol soil were mounted in trays and were treated monthly with 100 mL aliquots of DU solutions. Each set of 10 pots was treated exclusively with one of three doses comprising of 100, 250 and 400 mg/kg DU solution at 4-week intervals (Fig. 4-1). Another set of 10 pots filled with Pro-mixed peat was mounted in trays and treated monthly with a 250 mg/kg dose of depleted U. The pots were fertilized according to the fertilizer regime recommended for black spruce seedlings. The substrates used as experimental control were not treated with depleted U.

Prior to seedling transplant and DU treatments, the pots were watered with deionized water to 70-80% field moisture capacity and allowed to equilibrate for 21 days. Two-year-old seedlings of black spruce were transplanted from the potting compost into the experimental substrates and the controls (non-spiked substrates) and were periodically watered. The plants were grown in a greenhouse under a natural summer day/night regime, and were harvested after 8 weeks of growth. The above-substrate portions of the plants were excised with a pair of Teflon-coated shears to avoid contamination by DU-enriched soil particles.

A hydroponic setup representing control substrate containing only depleted U (20 ppm) was included. Prior to transplanting, all adhering potting material was removed from the root system of the seedlings by gentle agitation in deionized water. The roots were rinsed a second time by gentle swirling in beakers containing fresh deionized water, following which they were transferred to 100 mL beakers containing 80 mL aliquots of

the hydroponic nutrient solution (Peters Professional 5-11-26 Hydro-sol waters soluble fertilizer). Table 4-3 presents the major and minor plant nutrient composition of the hydroponic solution. The pH of the nutrient solution was adjusted to 7.4 with dilute solutions of HCl and KOH.

Table 4- 3: Plant nutrient composition of Peters Professional 5-11-26 nutrient solution used for the hydroponic study of U uptake by black spruce seedlings.

Nutrient	%
Total N	5
Available phosphate (P_2O_5)	11
Soluble potash (K_2O)	26
Mg total	3.1
S	4.0
B	0.05
Cu	0.015
Fe	0.3
Mn	0.05
Mo	0.01
Zn	0.015

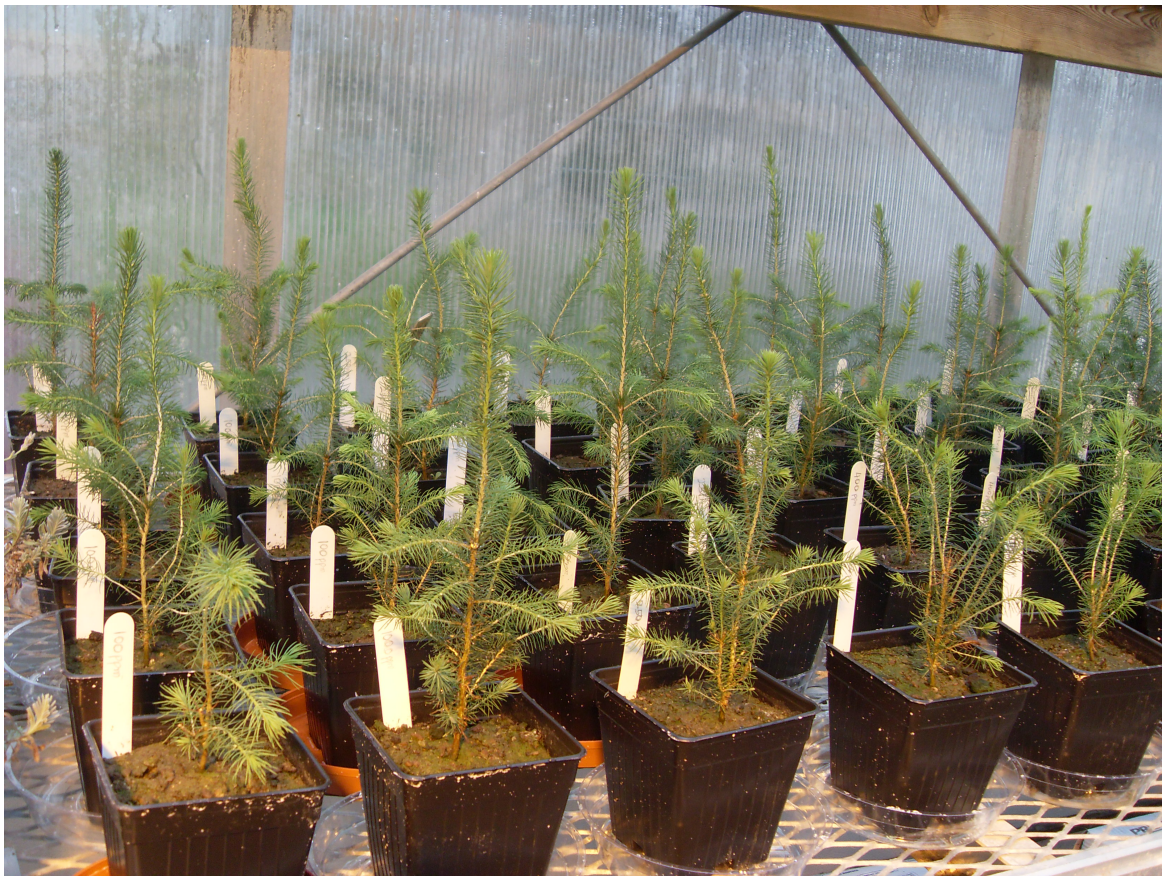

Figure 4- 1: Black spruce seedlings grown in pots treated with different doses of depleted uranium solution to investigate the true source of uptake of the metal by the plant root system. Doses comprised of 100 mL aliquotes of 100 ppm, 250 ppm, and 400 ppm of depleted uranium (uranyl nitrate hexahydrate) applied at 4 weeks intervals over an 8-week period. Each set of 10 pots was treated exclusively with one unique dose by adding the 100 mL aliquots in trays in which the pots were mounted.

Nutrient solutions in the hydroponic setup were periodically brought up to 80 mL to maintain a constant volume, and to compensate for evaporation. The seedlings were transferred into fresh nutrient solutions weekly to ensure an adequate nutrient supply and increased aeration. The stems of the seedlings were separated from the root systems after 8 weeks of growth and were rinsed twice with deionized water to remove any traces of surface DU contamination and dust particles.

4-2-3 Preparation and analyses of samples

The soil and peat samples (DU-spike and controls) oven-dried to constant weight were sieved through a 250 μm mesh. An approximate 100 mg aliquot of each soil sample was digested using 40% HF/8 mol/L HNO_3 (1:1 v/v) followed by aqua regia. Accurately weighted 1.0 g aliquots of peat (control and DU-treated) were ignited at 550 ^{o}C and the ash was dissolved in 2 mL of 40% HF/8 mol/L HNO_3 (1:1 v/v). The solutions were diluted gravimetrically to 60 g for determination of U isotopes using inductively coupled plasma quadrupole mass spectrometry (ICP-QMS) at the CREAIT facility of Memorial University of Newfoundland, St John's and a subsample analyzed by Neutron Activation Analysis (NAA) at the Becquerel Laboratories Inc., Ontario, Canada.

The extractable and residual portions of the substrates were obtained using 1 mol/L ammonium acetate (buffered at pH 4.5) and HF/HNO_3 leach, respectively. The in-house natural uranium standards (granite and uraninite ore) digested using the same analytical protocol as the soil samples were used to assess the accuracy and precision of the U isotope measurements. Black spruce stems were oven-dried at 75 ^{o}C to constant

weight and then pulverized into a fine powder. Approximately 1.0 g aliquot of the powdered material was ignited at 450 ^{o}C. The ash dissolved in 2 mL of 8 mol/L HNO_3 and gravimetrically diluted to 60 g for the determination of ^{235}U and ^{238}U. Each batch of ICP-QMS analysis included a four-element spike (Be, Tl, Pb, and Bi) solution, in-house granite and uraninite standards, and a reagent blank interspersed regularly between the samples. Data acquired were processed to correct for sensitivity and zero point charges using the monitors and blanks; matrix suppression and enhancement effects were corrected using Bi as an internal standard.

4-2-4 OPTIMIZATION OF THE INSTRUMENT FOR U ISOTOPE ANALYSIS

The precision of U isotope ratio analyses using ICP-QMS is primarily limited by the counting statistics for the lower abundance isotope (^{235}U) and instrument stability (Chenery, 2002). The detector system of the ICP-QMS was set to produce a signal of approximately 100,000 cps for the ^{235}U isotope in a 2 μg/L in-house granite standard solution. Analyte solutions were diluted such that the ^{235}U and ^{238}U signals were obtained in the pulse counting mode to avoid errors related to the cross calibration of digital to analogue modes. A series of dwell times (times at which the mass spectrometer acquires data at a particular mass) were studied and dwell times of 5 ms and 3 ms were chosen for the ^{235}U and ^{238}U respectively. A dead time of 50 ns was selected after a series of variations and measurements of isotope ratios was obtained. The dead time correction was applied off line.

4-2-5 ACCURACY AND PRECISION OF U ISOTOPE RATIO MEASUREMENTS

The uncertainty associated with ICP-QMS determination of the U isotopes is attributed to counting statistics of the blank and sample signals (Rosamilia et al., 2004). The gross intensities for the ICP-QMS determination of isotopes ^{235}U and ^{238}U were first blank-corrected with the mean intensity of the reagent blanks in each run. The $^{238}U/^{235}U$ ratios were then calculated as the ratio of the blank corrected intensities. These ratios were corrected for instrumental mass discrimination/bias by multiplying the blank corrected ratios by the ratio of 137.88 to the mean ($^{238}U/^{235}U$) ratio of the in-house granite and uraninite standards in that run. Assuming the natural and accepted $^{238}U/^{235}U$ ratio of 137.88.

Precision is expressed as the standard deviation for a single determination of isotopes ^{235}U and ^{238}U in the in-house reference materials. Uncertainty in the measurements of the isotopic ratios is related to the abundance of the isotope in the sample and is controlled to a large extent by Poisson counting statistics. The precision and accuracy of the mean isotopic ratios in the reference samples were estimated based on replicate measurements of the in-house reference standards. Errors inherent in replicate determinations of the $^{238}U/^{235}U$ ratios over six independent measurements are presented as two sigma standard deviations (2σ) (Fig. 4-2).

4-3 RESULTS AND DISCUSSION

4.3.1 URANIUM CONCENTRATION AND ISOTOPIC MEASUREMENTS

The instrument mass bias for ICP-QMS determination of U concentration and ^{235}U and ^{238}U intensities was monitored by analyzing reagent blanks and the in-house reference solutions (granite and uraninite) after every other sample tube. But for the high relative standard deviation of 0.40% in one of the granite determinations, the precisions for $^{238}U/^{235}U$ measurements of the in-house granite and uraninite ore reference samples were $\leq 0.2\%$. The range of ^{238}U to ^{235}U ratios in six replicate determinations of 2 μg/L granite and uraninite were, respectively, 137.18-138.42 and 137.64-138.30 (Fig. 4-2). Figure 4.2 highlights the mean $^{238}U/^{235}U$ ratio in the in-house reference materials and in soil and peat samples used as experimental controls.

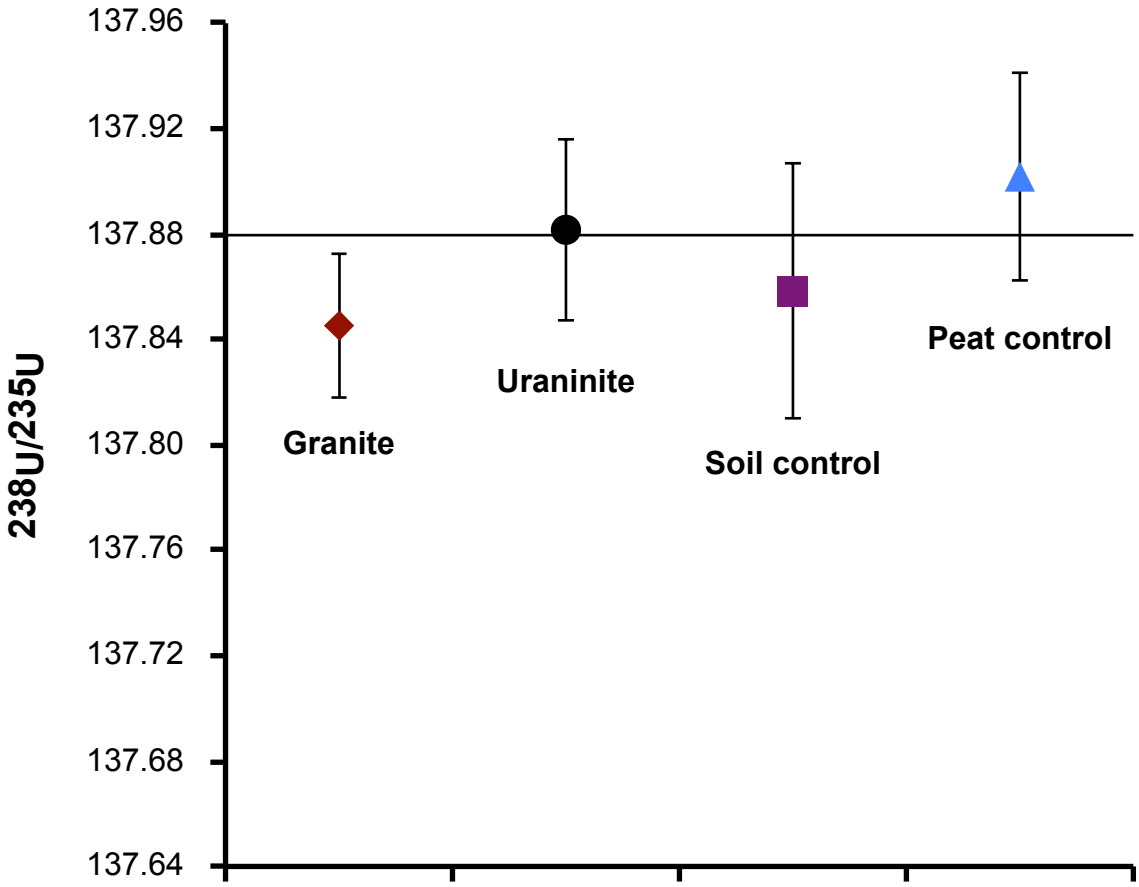

Figure 4-2: Mean $^{238}U/^{235}U$ ratios in in-house granite and uraninite reference materials and the experimental control substrates (soil and peat) analyzed with ICP-QMS. The error bars denote 2σ standard deviations for a single determination of the $^{238}U/^{235}U$ ratio in the in-house reference materials and uncontaminated substrates.

Uranium concentrations in the stems of spruce seedlings grown in pots treated with varying doses of DU increased linearly with the increasing concentration of the extractable fraction (1 mol/L ammonium acetate portion) of the substrates. For example, the mean U content in the stems increased from 4.3 ppm in plants grown in control pots

containing non-treated soil to 72.6 ppm in stems of plants grown in soils treated with 400 ppm of depleted U. Similarly, U concentrations in the extractable fractions of both the control and soil samples dosed with 400 ppm DU were 1.3 ppm and 28.6 ppm respectively (Appendix 4-2). The pattern of U accumulation in the roots, stems, and needles of black spruce seedlings was demonstrated in a previous study (Nyade et al., 2013a).

Deviations of the $^{238}U/^{235}U$ ratio from the accepted equilibrium value (i.e. 137.88) in environmental and/or geological samples suggest possible mixing of natural and non-natural U specifically, enriched and/or depleted uranium. The magnitude of the deviation is a function of the extent of enrichment of the ^{235}U isotope (Westphal et al., 2004; Sahoo, 2009) and the concentration present in the media. For example, the nuclear power industry enriches uranium to approximately 3.5% ^{235}U (low enriched U), whereas weapons and some test reactors may enrich to >90% ^{235}U (highly enriched U) (Ziegler and Bushaw, 2008). The waste streams from enrichment processes yield depleted U with lower abundances of the ^{235}U and ^{234}U isotopes (Sahoo, 2009).

Figure 4-3 shows a general increase in $^{238}U/^{235}U$ ratio of a subsample of substrates treated with different doses of DU whereas the isotopic ratio the experimental controls (i.e. soil and peat) approximated to the accepted equilibrium ratio in natural U. The NAA determinations of the ^{235}U isotope were generally characterized by fairly high uncertainties due to the relatively low natural abundance of this isotope. In particular, the standard deviations for NAA determinations of $^{235}U/^{238}U$ ratio in the DU-dosed substrates ranged from 7%-14% whereas the ICP-QMS determinations of $^{238}U/^{235}U$ isotope ratio in

both control and DU-treated samples had precisions ranging from 0.06% to 2.0%. The NAA technique therefore still fingerprinted the incidence of anthropogenic U contaminations in the DU-dosed soil samples, albeit with high uncertainty. Mitchel et al. (1999) reported moderate to good precision for the determination of U isotope composition and total concentration in geological and biological samples (e.g. U ore, urine, and blood) using the NAA technique. Although poor in precision with regard to U isotope measurements, the NAA technique lent itself to simple sample preparation and minimal contaminations from reagents and laboratory environment, since the technique does not involve sample preparation such as acid digestion (Miller, 2007).

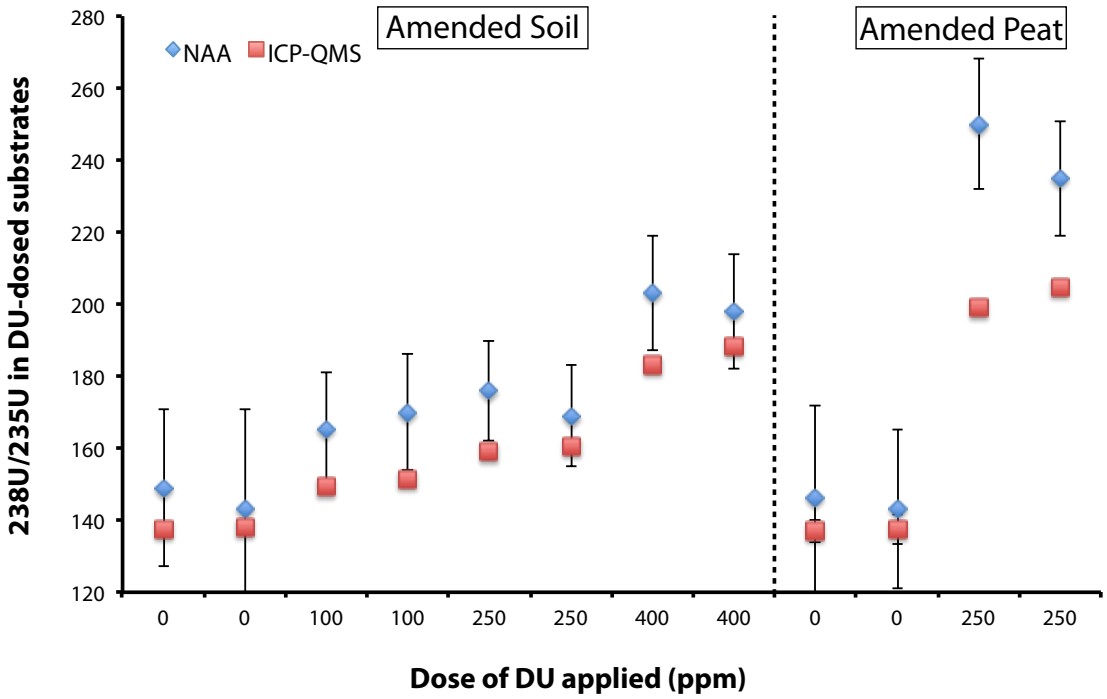

Figure 4- 3: The ratio ^{238}U to ^{235}U in HF/HNO_3-aqua regia portion of substrates treated with varied doses of DU and the experimental controls and analyzed using NAA and ICP-QMS.

Both the ICP-QMS and NAA measurements of the $^{238}U/^{235}U$ ratio in the 250 ppm dosed peat were found to be elevated relative to the ratios determined in soil treated with 250 ppm DU (Fig. 4-3). This may be due in part to the generally low concentration of natural uranium in the untreated peat and to the consequent challenge in detecting the low abundance isotope. However, compared to the multi-collector-inductively coupled plasma-mass spectroscopy and thermal ionization mass spectrometry (TIMS) known to produce high precision isotope measurements (Becker, 2005; Stirling et al., 2005; Weyer

et al., 2005), the quadrupole-based technique has superior sample throughput (up to 100 samples/day) accompanied by lower analytical costs. Becker and Dietze (1999) and Esaka et al. (2011) demonstrated high precision U isotope measurements in 1 μg/L U solutions and individual uranium-plutonium mixed oxide particles respectively, using quadrupole-based ICP-MS. In particular, Becker et al. (1999) reported precisions ≤0.03% for the $^{238}U/^{235}U$ isotopic ratio in a 1.0 μg/L U solution.

An alternative approach, the multi-collector-inductively coupled plasma-mass spectroscopy (MC-ICP-MS) has advantages over ICP-QMS and TIMS, especially when a Faraday detector is used to collect the high abundance ^{238}U isotope, whereas the low intensity isotopes (^{234}U and ^{235}U) can be collected in ion multiplier channels equipped with ion counting systems. Weyer and co-workers (2008) established high precision isotope variations ($^{238}U/^{235}U$) in natural geological samples (e.g. basalts, granites, seawater, corals, black shales, *etc*.) using MC-ICP-MS. Similarly, Stirling et al., (2007) resolved variations $^{238}U/^{235}U$ at the 0.4 epsilon level in sample sizes comprised of 50 ng U using multi-collector ICP-MS. That notwithstanding, the measurement of U isotopes using MC-ICP-MS is not without challenges, as the technique requires more expensive instrumentation. Though sample throughput in terms of sample preparation and analyte measurement are similar to quadrupole instruments, longer sample integration times are used when very low uncertainties are required, sometimes allowing fewer than 10 samples per day to be analyzed (Chenery et al., 2002; Becker 2005).

The uranium concentration in the stems of black spruce seedlings grown in DU-treated pots exhibited a close correlation with the doses applied to the substrates and

in particular, U content of the extractable fraction of the treated substrates. The source of U taken up by the spruce seedling was examined by comparing ICP-QMS measured $^{238}U/^{235}U$ in the extractable fraction of the DU-dosed substrates with the $^{238}U/^{235}U$ in the stems of seedlings grown in pots containing DU-treated substrates (Fig. 4-3). The measured $^{238}U/^{235}U$ exhibited a marked increase in both the extractable and the total digested portions (i.e. HF/HNO_3-aqua regia) of the DU-treated substrates as the dose increased. The isotopic ratios in the extractable fraction were, however, substantially elevated relative to the measured ratios in the total digested portions of the DU-dosed substrate - providing evidence of the leaching of the DU weakly adsorbed on to the mineral phases. Conversely, the decreased $^{238}U/^{235}U$ in the total digested portion is a consequence of apparent mixing of the leached natural U hitherto bound to the mineral phases (i.e. organics, oxides, and residuals) and the DU spike.

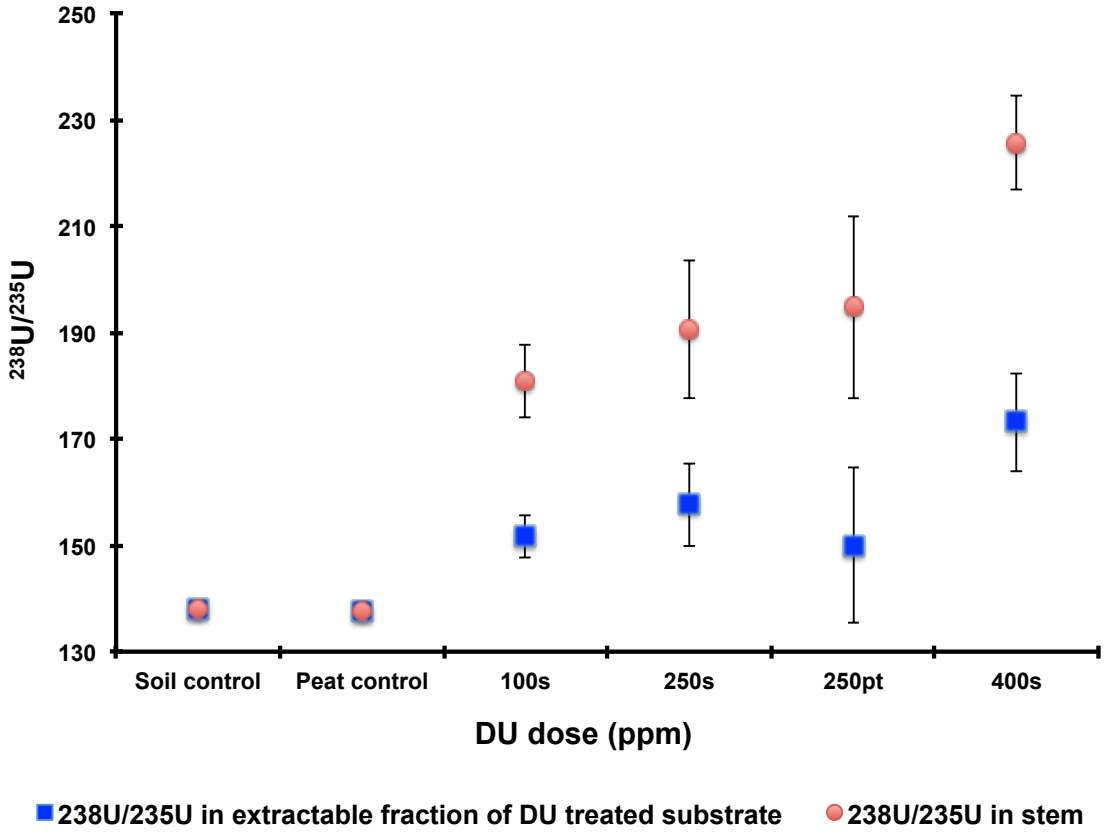

Figure 4-4: The relationship between mean $^{238}U/^{235}U$ ratio in the extractable (1 M ammonium acetate) portion of substrates treated with varied doses of DU and the isotopic ratios in stem of seedlings grown on the DU-dosed substrates. Error bars denote standard deviations for the individual determinations. (Substrate code: s = soil, pt = peat).

Likewise, the measured $^{238}U/^{235}U$ ratio in the stems of seedlings grown in DU-dosed pots exhibited a linear association with that in the extractable (bioavailable) portion of the substrates (Fig. 4-4), suggesting a linkage between U content in the plant tissues and dissolved DU in the solution spike. Indeed the elevated $^{238}U/^{235}U$ ratio (i.e. >137.88) reflected non-natural isotopic signatures traceable to the dissolved DU spike.

Thus, isotopic ratios of 174±5, 187±6, and 210±4 were measured in the stem of spruce seedlings grown in pots dosed with 100, 250, and 400 ppm DU, respectively. The deviation from the accepted natural $^{238}U/^{235}U$ ratio is a simple consequence of the disequilibrium caused by high uptake of the ^{235}U deficient-DU solution.

As expected, $^{238}U/^{235}U$ ratio in the stems of black spruce seedlings grown in control pots is consistent with the accepted natural ratio and ranged from 137.68-138.07 (mean of 137.84). The association of the $^{238}U/^{235}U$ ratio in seedlings with the ratio in the extractable portion of substrates (Fig. 4-4) demonstrates that a substantial amount of U accumulated by the black spruce seedlings is derived from the dissolved DU in the solution spike and taken up through the root system as evidenced by the significantly high U concentration in seedlings grown in pots dosed with DU solution relative to seedlings grown on control substrates.

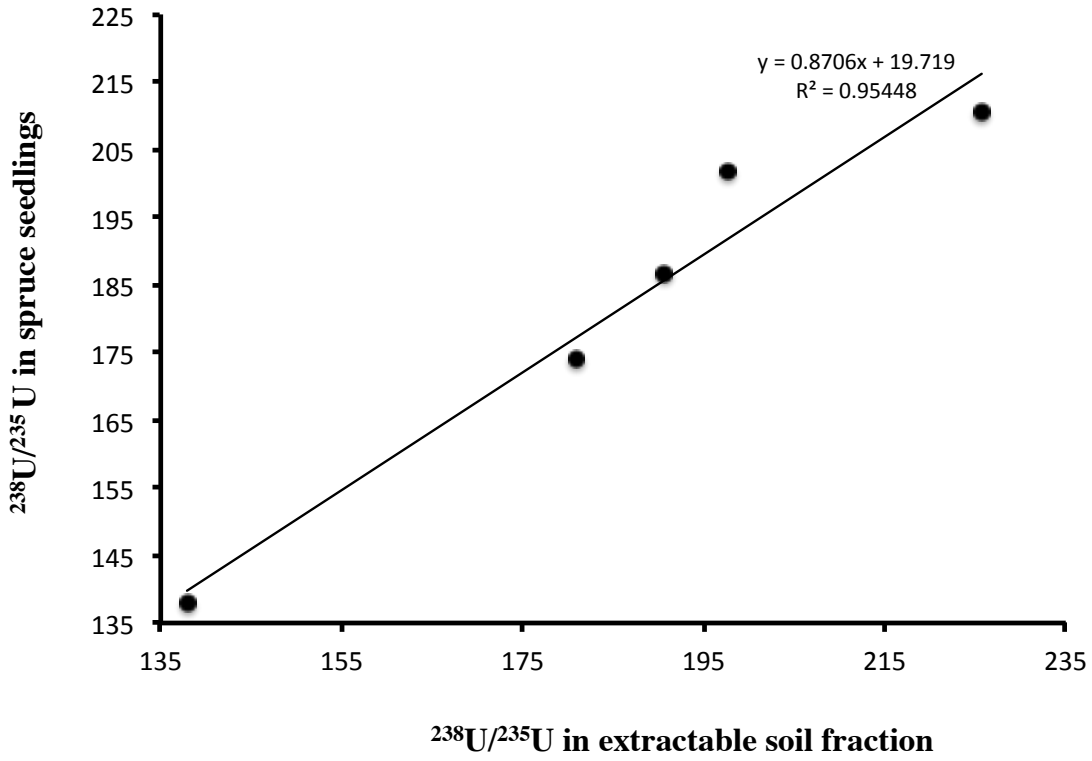

Figure 4-5: Relationship between mean $^{238}U/^{235}U$ ratio in spruce seedlings grown on DU-dosed soil and experimental control and the isotope ratio in the extractable fraction of the DU-dosed soil. The linear relationship suggests greater uptake of soluble depleted uranium otherwise weakly adsorbed to mineral phase rather than natural uranium in the soil.

The greenhouse study established that the measurement of $^{238}U/^{235}U$ ratio in spruce trees not only identifies the source of metals taken up by vegetation but is also an effective tracer of the incidence of non-natural U in groundwater, geological, and environmental samples as the DU signature of the DU-treated substrates manifested in the

plant tissues. Likewise, Yoshida et al. (2000) measured the $^{235}U/^{238}U$ ratio in soil and vegetation as a tracer of non-natural uranium contamination in the vicinity of a uranium conversion plant in Tokai-mura, Japan. The anthropogenic U detected in the vegetation was attributed to an uptake of enriched U from the contaminated soil and atmospheric fallouts of enriched U-bearing particulates. The latter is clearly discounted in this study since the seedlings were isolated (by the greenhouse) from any significant impact of atmospheric particulates during cultivation and the plant parts were thoroughly washed to remove any traces of DU containing precipitates on the stems prior to analysis.

The elevated $^{238}U/^{235}U$ ratio in seedlings grown in DU-dosed hydroponic nutrient solutions is consistent with the ratio in the DU-treated growth medium. The measured ratio in individual plants ranged from 358.8 to 366.13 (mean = 363.2±1.2) indicating an uptake of the dissolved DU. A Student t-test (95% CL) showed a statistically significant difference between the mean $^{238}U/^{235}U$ ratio (363.2±1.2) in the stems of seedlings grown in DU-dosed hydroponic nutrient solutions and the mean isotopic ratios of 150.2±0.4 and 201.8±0.5 (Fig. 4-6) determined in seedlings grown in DU-dosed soil and peat respectively (Davis, 2002). The relatively low $^{238}U/^{235}U$ ratio in soil and peat compared to the ratio determined in seedlings grown in DU-dosed hydroponic solutions is a consequence of isotopic mixing of natural U and the DU.

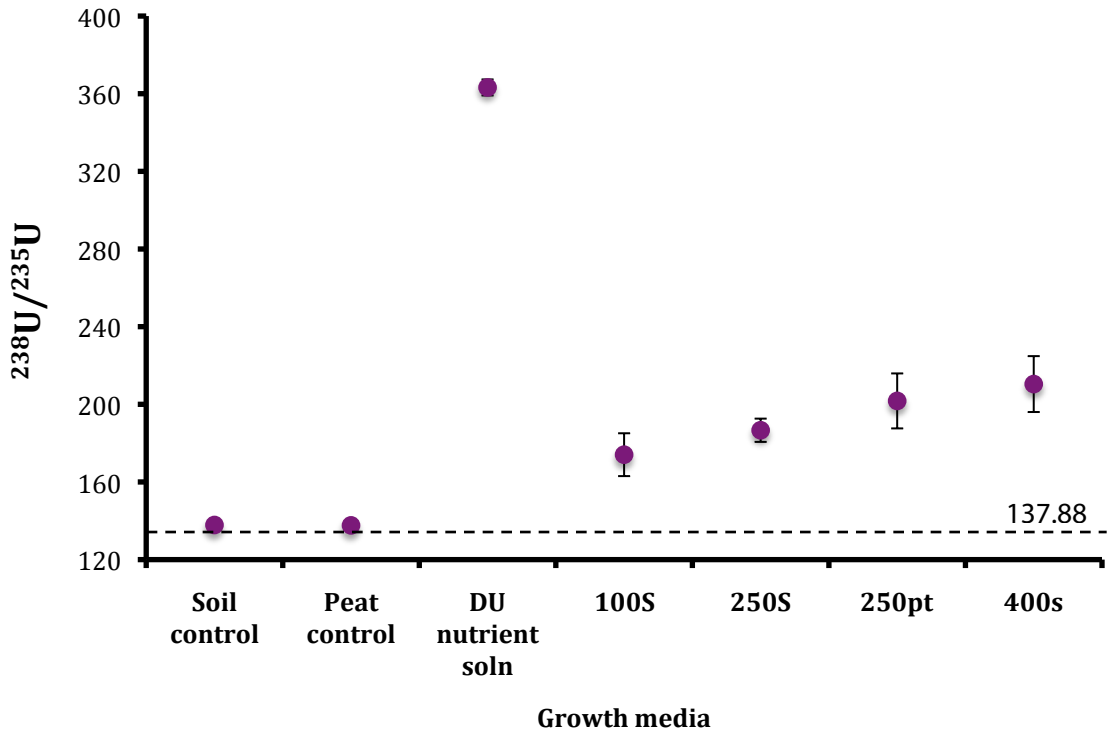

Figure 4-6: Mean $^{238}U/^{235}U$ ratios in two-year-old black spruce seedlings grown on peat, soil, and hydroponic solutions spiked with varying concentrations of depleted U. Control soil and peat are natural samples containing common U and are not contaminated with DU. DU nutrient "soln" consists of 100 mL aliquots of hydroponic solution spiked with 20 ppm of depleted U, while samples 100S, 250S and 400S are soil samples respectively dosed with 100 ppm, 250 ppm and 400 of depleted U. Sample 250pt is peat-treated 250 ppm of depleted U. Error bars denote standard deviations for the $^{238}U/^{235}U$ determinations.

Comparing the magnitude of U uptake and accumulation in seedlings and the extent of deviation of the $^{238}U/^{235}U$ ratio from the accepted natural ratio (137.88) as the dose of the DU spike increases suggest a greater uptake of dissolved DU relative to

absorption of natural uranium leached from mineral phases in soil or peat. The low uptake of natural U is explained by the sequential chemical extraction of the substrates (Nyade et al., 2013) and MLA-SEM analysis of the soil (Wilton, 2012), which revealed the sequestration of the natural U onto the oxides and residual (albite grains) phases in the soil, hence less available in labile forms for uptake by the plants. Accordingly, metals taken up by the plants are sourced predominantly from the dissolved components in water (i.e. groundwater, soil pore water and/or surface water accessible to plant roots) as opposed to U bound to mineral phases in the soil or lithosphere. This observation agrees with the results of a field sampling by Dunn (1980), where the U concentration in black spruce twigs were found to be unrelated to metal content in soil and bedrock but to the U-rich formation water moving upwards from depths of 60-250 m. The analysis of U isotopes in spruce trees therefore, not only has important implications for biogeochemical prospecting for concealed ore but to environmental biomonitoring, and groundwater quality assessments (Tripathi et al., 2013).

4-4 CONCLUSIONS

It has been established through the determination of $^{238}U/^{235}U$ ratio in stems of spruce seedlings grown on substrates treated with depleted U solutions that a substantial quantity of the U taken up by the plants is derived from the labile uranium component in water (i.e. groundwater and/or surface) rather than U adsorbed onto mineral phases in soil. Through the measurement of $^{238}U/^{235}U$ ratio, it has been possible to fingerprint the incidence of non-natural U contamination (depleted and/or enriched U) in soil,

groundwater, and the bedrock that interact with the plant root system. The accuracy and precision of the measured isotopic ratios were assessed by analyzing $^{238}U/^{235}U$ ratios in in-house granite and uraninite reference solutions, based on the assumption of an invariant $^{238}U/^{235}U$ ratio (137.88). The precision for ICP-QMS measurement of $^{238}U/^{235}U$ ratio in the DU-treated substrates and in stems of seedlings grown on the DU-treated media varied from 0.02 to 2.7 and 0.05 to1.9, respectively. Isotopic measurements using NAA were characterized by higher uncertainties (i.e. relative standard deviations ranged from 7–14%).

ICP-QMS analysis of the $^{238}U/^{235}U$ ratio in the stems of spruce seedling grown on DU-dosed media showed significant variations in the $^{238}U/^{235}U$ ratios relative to a mean ratio of 137.86 determined in seedlings grown in the control soil samples. The $^{238}U/^{235}U$ ratio in soils increased linearly with increasing dose of DU in the solution spike. Similarly, mean $^{238}U/^{235}U$ ratios of 174±5, 187±6, and 210±4 were detected in the stems of seedlings grown in pots treated with 100 ppm, 250 ppm, and 400 ppm DU. The elevated $^{238}U/^{235}U$ ratio in the seedlings suggests that the DU in the solution spike and the U content in the plant tissue are inextricably related. Thus metals taken up by vegetation occur via hydraulic lift by the plant root system of the dissolved metal ions in groundwater. This observation conclusively demonstrates that the hydrosphere acts as an essential and intermediate link in the transfer of uranium from the lithosphere to the biosphere. The plant biogeochemistry of an area, therefore, more importantly reflects the hydrogeochemistry rather than the soil geochemistry.

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References

Abdelouas, A. (2006). Uranium mill tailings: geochemistry, mineralogy, and environmental impact. Elements, 2: 335-341.

Alfani, A.; Bartoli, G.; Rutigliano, F.A.; Maisto, G.; Virzo, and De Santo, A. (1996). Trace metal biomonitoring in soil and the leaves of *Quercus ilex* in the urban area of Naples. Biological Trace Element Research, 51: 117-131.

Becker, J.S. (2005). Recent developments in isotope analysis by advance mass spectrometry techniques. Journal of Analytical and Atomic spectrometry, 20: 1173-1184.

Becker, J.S. and Dietze, H-J (1999). Precise isotope ratio measurements for uranium, thorium and plutonium by quadrupole-base inductively coupled plasma mass-spectrometry. Fresenius Journal of Analytical Chemistry, 236: 482-488.

Boulyga, S.F. and Becker, S.J. (2002). Isotope analysis of uranium and plutonium using ICP-MS and estimation of burn-up of spent uranium in contaminated environmental samples. Journal of Atomic Spectrometry, 17: 1143-1147.

Cannon, H.L. (1961). Botanical prospecting for ore. Virginia Miner, 7(1): 1-11

Chen, J.H. and Wasserbug, G.H. (1981). Isotopic determination of uranium in picomole and subpicomole quantities. Analytical Chemistry, 53: 2060-2067.

Chenery, S.R.N.; Ander E.L.; Perkins K.M. and Smith B. (2002). Uranium anomalies identified using G-BASE data – Natural or anthropogenic? A uranium isotope pilot study. Internal report IR/02-001, British Geological Survey.

Davis, J.C. (2002). Statistics and data analysis in geology, (3rd Edn). John Wiley and Sons, Toronto, pp 60-74.

Di Guardo, A.; Mariani, G.; Guzzi, A.; Fanelli, R. and Calamari, D. (1999). Field derived BCFs in pine needles for the calculation of air concentration of dioxin. Organohalogen Compounds, 43, 275-278.

Di Guardo, A.; Zaccara, S.; Cerbolini, B.; Acciarri, M.; Trezaghi, G. and Calmari D., (2003). Conifer needles as passive biomonitors of the spatial and temporal distribution of DDT from a point source. Chemosphere, 52: 789-797.

Dunn, C.E. (1981). The biogeochemical expression of deeply buried uranium mineralization in Saskatchewan, Canada. Journal of Geochemical Exploration, 15: 437-452.

Dunn, C.E. (2007). Biogeochemistry in mineral exploration. Elsevier, Amsterdam, Vol. 9.

Esaka, F.; Magara, M.; Suzuki, D.; Miyamot, Y.; Lee C-G. and Kimura, T., (2011). Feasibility study of isotope ratio analysis of individual uranium plutonium mixed oxide particles with SIMS and ICP-MS. Mass Spectrometry Letters, 2(4): 80-83.

Margui, E.; Queralt, I.; Carvalho, M.L.; and Hidalgo, M. (2005) Comparison of EDXRF and ICP-OES after microwave digestion of element determination in plant specimen from an abandoned mining area. Analytica Chimica Acta, 549: 197-204.

Markert, B.; Wappelhorst, O.; Weckert, V.; Siewers, U.; Friese, K. and Breulmann, G. (1999). The use of bioindicators for monitoringthe heavy-metal status of the environment. Journal of Radioanalytical and Nuclear Chemistry, 240(2): 425-429.

Miller, A.C. (2007). Properties, uses, and health consequences. CRC Press, London, pp 121-142.

Mitchel, R.E.J.; Jackson, J.S.; and Helmiller, C. (1999). Inhaled uranium ore dust and lung cancer risk in rats. Health Physics, 72(2): 145-155.

Normandin, L.; Kennedy, G.; and Zayed, J. (1999). Potential of dandelion (*Taraxacum officinale*) as a bioindicator of manganese arising from the use of methylcyclopentadienyl manganese tricarbonyl in unleaded gasoline. The Science of the Total Environment, 239: 165-171.

Nyade, P.; Wilton D.; Longerich, H.; Thompson, G.; and McNeill, P. (2013a). Evaluation of uranium accumulation in Black spruce trees. Research Journal of Environmental and Earth Sciences, 5(3): 113-122.

Nyade, P.; Wilton, D.; Longerich, H.; Thompson, G., and McNeill P. (2013b). Use of surficial geochemical methods to locate areas of buried uranium mineralization in the Jacque's Lake area of the Central Mineral Belt, Labrador, Canada. Canadian Journal of Earth Sciences. dx.doi.org/10.1139/cjes-2013-0032.

Rogstad, S.H.; Keane, B.; Collier, M.; and Sann Joi (2002). Plant biomonitors: pollution, dandelions, and mutation rates In Chemicals in the environment. American Chemical Society Series, 806: 350-362.

Rosamilia, S.; Gaudino, S.; Sansone, U.; Belli, M.; Jeran Z.; Ruisi, S. and Zucconi, L. (2004). Uranium isotopes, metal and other elements in lichens and tree bark collected in Bosnia-Herzegovina. Journal of Atmospheric Chemistry, 49: 447-460.

Sahoo, S.K. (2009). Measurement of uranium and its isotopes at trace levels in environmental samples using mass spectrometry. Indian Journal of Physics, 83(6): 787-797.

Tripathi, R.M.; Sahoo, S.K.; Mohapatra, S.; Lenka, P.; Dubey, J.S. and Puranik, V.D. (2013). Study of uranium isotopic composition in groundwater and deviation from secular equilibrium condition. Journal of Radioanalytical and Nuclear Chemistry, 295: 1195-1200.

Stirling, C.H.; Andersen, M.B.; Potter, E-K and Halliday, A.N. (2007). Low-temperature isotopic fractionation of uranium. Earth and Planetary Science Letters, 264: 208-225.

Stirling, C.H.; Halliday, A.N. and Porceli, D., (2005). In search of live ^{247}Cm in early solar systems. Geochemica et Cosmoschimica Acta, 69(4): 1059-1071.

Tremolada, P.; Burnett V.; Calmari D. and Jones K.C. (1996). A study of the spatial distribution of PCBs in the UK atmosphere using pine needles. Chemosphere 32: 2189-2203.

Westphal, C.S.; McLean, J.A.; Hakspiel, S.J.; Jackson, W.E.; McClain, D.E., and Montaser, A. (2004). Determination of depleted uranium in urine via isotope ratio measurements using large-bore direct injection high efficiency nebulizer coupled plasma mass spectrometry. Applied Spectroscopy, 58(9): 1044-1050.

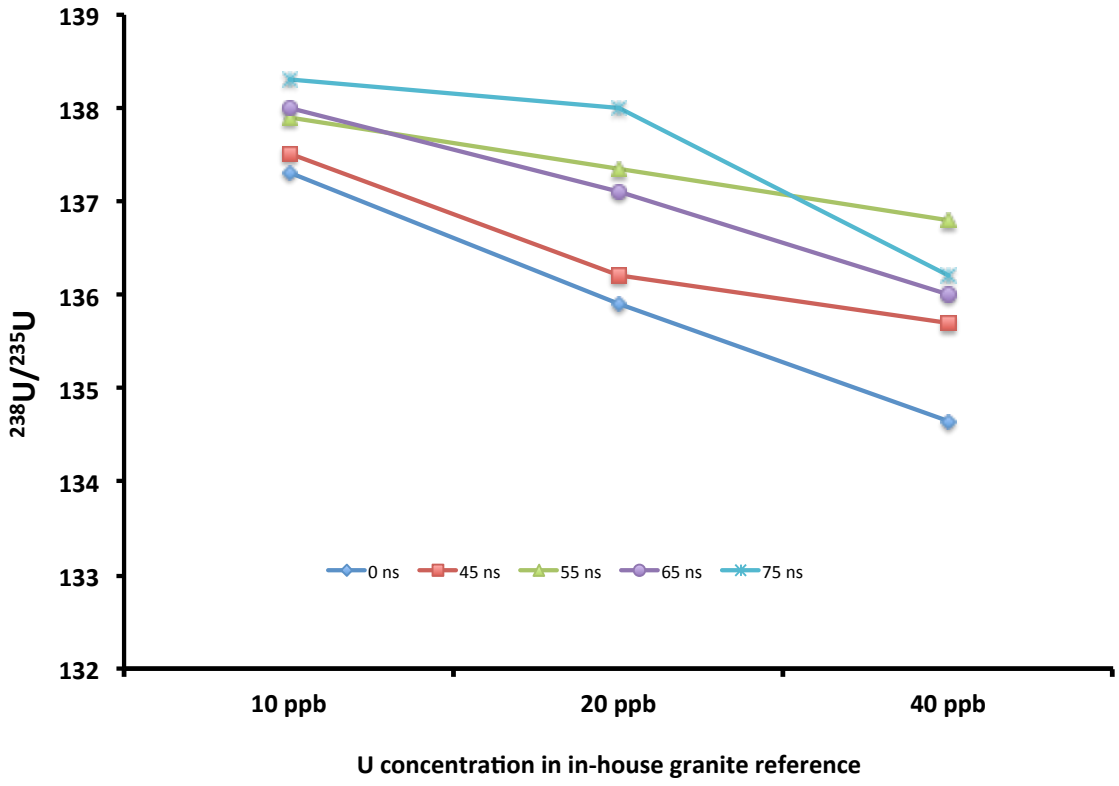
Weyer, S.; Anbar, A.D.; Gerdes, A.; Gordon, G.W.; Algeo, T.J. and Boyle, E.A. (2008). Natural fractionation of 238U/235U. Geochimica et Cosmochimica Acta, 72: 345-359.

Yoshida, S.; Muramtsu, Y.; Tagami, K.; Uchida, S.; Ban-nai, T.; Yonehara, H. and Sahoo, S. (2002). Concentrations of uranium and 235U/238U ratios in soil and plant samples collected around the uranium conversion building in the JCO campus. Journal of Environmental Radioactivity, 50: 162-172.

Ziegler, S.L. and Bushaw, B.A., (2008). Ultratrace uranium fingerprinting with isotope selective laser ionization spectrometry. Analytical Chemistry, 80: 6029-6033.

Appendix 4- 1: Optimization of Dead Time for $^{238}U/^{235}U$ using the ICP-QMS

The dead time was optimized according to the method proposed by Chenery et al. (2002). The optimum dead time was derived by analyzing $^{238}U/^{235}U$ ratios in serially diluted solutions of the in-house granite reference sample with concentrations varying from 10 to 40 ppb and analyzed at dead time correction values of 0, 45, 55, 65 and 75 ns. The dead time correction value for which the $^{238}U/^{235}U$ ratio was stable (refer to figure below) and closest to the true value at all concentrations was chosen as the optimum dead time correction value. Consequently, a dead-time value of 55 ns was chosen.



References

Chenery, S.R.N., Ander E.L., Perkins K.M., Smith B. (2002). Uranium anomalies identified using G-BASE data – Natural or anthropogenic? A uranium isotope pilot study. Internal report IR/02-001, British Geological Survey.

Appendix 4- 2: Uranium concentration in the extractable fraction of DU-dosed soil and the metal content in stems of seedlings grown in the amended substrates

Dose of DU in soil (ppm)	Mean U in stems of seedling	Mean U in extractable fraction of DU-amended soil
Control (0 ppm)	4.3	1.3
100	17.1	11.4
250	41	18.6
400	72.6	28.6

Appendix 4- 3: The ratio of ^{238}U to ^{235}U in the HF/HNO_3-aqua regia portion of substrates treated with varied doses of DU and the experimental controls and analyzed using NAA and ICP-QMS

Substrates	Treatment with DU (ppm)	NAA	RSD	ICP-QMS	RSD
		$^{238}U/^{235}U$	%	$^{238}U/^{235}U$	%
C-soil-01	0	149	11	137.7	0.15
C-soil-02	0	143	14	138.0	0.2
100S-01	100	165	8	149.2	0.5
100S-02	100	170	8	151.2	0.2
250S-02	250	176	7	158.9	0.9
250S-03	250	169	7	160.6	0.4
400s-01	400	203	8	183.2	0.07
400S-03	400	198	8	188.0	0.08
C-peat-01	0	146	13	136.9	1.5
C-peat-02	0	143	11	137.4	2.0
Peat250-02	250	250	9	198.8	0.3
Peat250-03	250	235	8	204.8	0.06

Appendix 4- 4: Uranium isotope ratios ($^{238}U/^{235}U$) of in-house granite and uraninite reference materials

Run	$^{238}U/^{235}U$ Granite	$^{238}U/^{235}U$ Uraninite
1	137.96±0.11	138.39±0.15
2	137.18±0.09	137.69±0.11
3	138.42±0.17	138.07±0.08
4	137.83±0.09	137.64±0.2o
5	137.93±0.12	137.82±0.18
6	137.75±0.40	137.77±0.11
Mean	137.85	137.90
σ	0.40	0.26
SEM	0.163	0.103

Appendix 4- 5: $^{238}U/^{235}U$ ratios in DU-spiked substrates digested using aqua regia for total decomposition

Replicates	Soil control	Peat control	100s	250s	250pt	400s
1	137.9	138.0	154.0	159.2	141.6	171.4
2	138.3	137.9	151.8	158.5	150.7	178.8
3	137.5	137.7	148.9	157.4	160.1	179.5
4	137.8	136.7	150.3	156.6	147.9	171.6
5	137.2	137.8	154.0	161.6	157.7	172.4
6	138.4	138.2	149.8	149.8	151.6	166.1
7	138.1	137.4	153.0	160.9	140.9	172.1
Mean	137.9	137.7	151.7	157.7	150.1	173.1
σ	0.4	0.5	2.04	3.9	7.3	4.6
sem	0.17	0.18	0.77	1.48	2.77	1.76

Appendix 4- 6: $^{238}U/^{235}U$ ratio in the extractable fraction of soil and peat treated with varied concentration of depleted uranium

Replicates	Soil control	Peat control	100s	250s	250pt	400s
1	139.0	138.0	184.0	191.6	197.7	225.4
2	137.3	135.9	179.8	201.8	187.3	221.6
3	138.0	138.0	174.0	191.6	197.7	227.8
4	137.9	138.3	181.8	188.6	196.6	230.7
5	138.0	138.0	184.0	191.6	207.7	231.5
6	137.1	138.1	180.8	179.6	192.0	219.7
7	137.9	137.7	182.4	189.6	204.8	223.6
Mean	137.9	137.7	181.0	190.6	197.7	225.7
σ	0.6	0.8	3.4	6.5	6.9	4.5
sem	0.23	0.31	1.30	2.44	2.64	1.69

Appendix 4- 7: Uranium isotope ratios in stems of black spruce seedlings grown in pots treated with varied doses of depleted uranium

Replicates	Soil control	Peat control	DU nutrient solution	100S	250S	250pt	400s
1	138.0	138.6	367.6	184.0	191.6	197.7	221.4
2	138.4	138.0	361.6	183.0	190.9	197.9	221.6
3	137.3	137.0	357.1	159.8	175.0	209.1	193.7
4	137.5	137.5	365.0	166.8	187.5	222.0	192.4
5	139.0	138.0	368.7	1834.0	191.3	197.7	221.5
6	137.3	137.9	359.1	179.8	190.7	177.3	220.9
7	136.9	138.3	366.0	181.8	188.6	196.6	221.7
8	137.1	136.9	359.3	158.8	179.8	201.9	193.3
9	140.1	137.5	367.1	160.8	180.6	225.7	195.1
10	137.1	137.1	360.8	180.8	189.6	192.0	221.6
mean	137.8	137.6	363.2	174.1	186.8	201.8	210.4
σ	1.0	0.5	4.1	11.0	6.0	14.2	14.4
sem	0.32	0.16	1.31	3.49	1.90	4.48	4.55

Chapter 5

Conclusions

The ultimate goal of this study was to explore the usefulness of black spruce trees as a suitable biogeochemical sampling medium and as an effective tool for the environmental monitoring of radionuclide contamination. A biogeochemical study, the first in Labrador, explored the effectiveness of vegetation analysis at locating buried mineralization targets in a till covered terrain. The case study compared the biogeochemistry of vegetation; the geochemistry of <250 μm sized-fraction of B-horizon soil, and the U/Th responses from an airborne radiometric survey to delineate concealed mineralizations at the Jaceque's Lake area of the Central Mineral Belt (CMB), Labrador. The second aspect of the thesis involved studies of U uptake by black spruce trees in a controlled greenhouse environment. The greenhouse study also evaluated the true source of metals taken up by vegetation and their usefulness in fingerprinting the incidence of radionuclide contamination in the soil and/ or groundwater. Thus, whether the metals were derived directly from the surface soil or till overburden or were dissolved ions in groundwater and taken up by plant root system.

The second chapter analyzed black spruce twigs, bark and needles, and soil geochemistry of the <250 μm fraction of B-horizon soil to outline surface signatures of the buried U mineralization in the Jacque's Lake area of the CMB. The till overburden at the Jacque's Lake area presents challenges to the conventional geochemical exploration methods because they effectively inhibit the direct observation of mineralized outcrops and accompanying alteration haloes. Further, the rugged topography and a dense

coniferous forest cover limit the effective application of geological and geophysical methods for prospecting the buried ore.

This study was based on airborne helicopter sampling of black spruce twigs with needles (i.e., 7 – 10-year-old twigs) and ground level sampling of black spruce bark, Labrador tea (leaves and stems), and B-horizon soil to locate areas of anomalous uranium mineralization and related pathfinder elements. Samples were collected along NE-oriented grid laid out perpendicular to the detected U/Th anomalies that define the Jacque's Lake prospect to allow for comparison between the surficial methods and the radiometric responses. Multi-element analysis of the vegetation, in particular, spruce twigs and bark delineated zones of anomalous U enrichment. Mean U concentrations in vegetation were of the order: spruce twigs > spruce stem outer bark > Labrador tea stem > spruce needles > Labrador tea leaves. Spatial plots of U concentrations in spruce twig ash however, discriminated the greatest anomaly to background contrasts and outlined the detected U/Th anomalies more precisely.

Binary plots and multivariate analyses of the biogeochemical data delineated the buried U mineralization along with Pb, Ag, Sb, Ce, Be, Tl, and Ca as the pathfinder elements in the spruce twig samples. Typically, the metallic elements showed varied patterns of enrichment in the individual plant parts. The biogeochemical data showed a high enrichment of Al, Mg, Rb, Zn, Mn, and Cd in ash of the bark of black spruce trees. The U contents of spruce twigs, outer bark, needles, Labrador tea stem and leaves denoted by the median and maximum concentrations were respectively 17 and 61 ppm; 3.1 and 23 ppm, 0.1 and 0.3 ppm, 1.4 and 8 ppm; and 0.04 and 0.85 ppm.

Spatial plots of the biogeochemical data established that areas with elevated U concentrations in twigs occur in the vicinity of the northeasterly-trending fault structures where mineral-enriched groundwater is discharged. Element enrichments in black spruce needles and Labrador tea leaves and stems were generally very low. To the contrary, the black spruce bark samples, constrained useful information relating to the buried mineralization, although characterized by relatively low element enrichment.

Uranium concentrations in the sub-250 μm size fraction of B-horizon soil were generally very low, except in two samples where anomalously high concentrations were detected. Spatial plots of U contents in the sub-250 μm size fraction exhibited discrete patterns of enrichments that were uncorrelated to the detected U/Th anomalies that characterize the Jacque's Lake prospect. Areas with elevated U anomalies appeared easterly displaced relative to NE-trending U/Th responses, suggesting a sub-vertical migration of mobile mineral constituents from the buried mineralization and/ or lateral dispersion via hydromorphic processes. In summary, plant analysis proved to be a viable technique for delineating U mineralization concealed by varying depths of exogenous material. Black spruce twigs are considered the most suitable sampling media because they produced greater anomaly to background contrasts and outlined the buried mineralization more precisely than B-horizon soil. Compared to soil geochemistry in the glacial till-covered terrain, biogeochemical responses provided greater leads for further geological and geophysical investigation in the Jacque's Lake area of the CMB, Labrador.

The Chapter 3 investigated the U-accumulating potential of black spruce trees and concentration ratios in spruce trees. The study provided the first experimental data on U

uptake, translocation, and accumulation in parts of black spruce seedlings. The greenhouse experimental studies involved the examination of U uptake from substrates (soil and peat) dosed with varying concentrations of depleted uranium and the experimental control (i.e. non-contaminated substrates). Uranium accumulation in plants is assessed by the bioaccumulation coefficient defined as the ratio of the concentration of U in the plant (ug/g) to the bioavailable concentration in the substrates (ug/g).

The results established that U uptake by spruce trees is dependent on a number of abiotic factors, including, but not limited to, metal bioavailability and substrate composition, and plant-specific parameters. This is illustrated by the substantial differences in U concentrations determined in the stem and roots of seedlings grown in soil dosed with DU and seedlings grown on DU-treated peat. Seedlings grown in amended soil are generally more enriched with U due to high availability of the metal in soil, unlike in peat, where uranyl cations form stable complexes with humic substances.

Uranium content in roots, stems, and needles of seedlings grown on substrates treated with DU suggests preferential enrichment in stems and follows the order stems > roots > needles. The U enrichment in the individual plant parts increased linearly with the available concentration in the substrate and more importantly with increasing dose of the DU spike. Thus, the concentration in stems of seedlings grown in soil dosed with 100, 250, and 400 mg/kg of uranyl nitrate hexahydrate were respectively 10, 24, and 50-fold greater than the concentration found in the stems of seedlings grown in control soil. The bioaccumulation coefficients for U in roots and stems also displayed a progressive increase with increasing available concentrations in the substrates. In particular, the soil

to root transfer of U increased from 0.91 in seedlings grown in control soil to 2.77 in seedlings grown in pots dosed with 400 ppm of uranyl nitrate hexahydrate. The roots to stem transfer increased from 2.14 in control seedlings to 3.52 in seedlings grown on soil dosed with 400 ppm U. It is inferred based on U contents and accumulation ratios in roots and stems of black spruce seedlings grown in DU-treated substrates that the quantity of metal taken up is a function of the available concentration in the substrate.

Chapter four of this study focused on the measurements of U isotope ratios in black spruce seedlings to fingerprint the ultimate source of metals taken up by vegetation. The objective is to determine whether metals taken up by the plants were directly derived from mineral phases in soil or were dissolved ions in groundwater and absorbed by the plants. Depleted U is used as a tracer of dissolved U ions in water. Typically, a secular equilibrium exists between the major isotopes of natural U; however, isotopic mixing caused by non-natural U contamination can displace the natural isotopic ratio.

The uptake of the major isotopes of U (^{238}U and ^{235}U) by black spruce seedlings grown in substrates dosed with varied concentrations of depleted uranium was examined in a controlled greenhouse experiment. The results of ICP-QMS and NAA determinations of the $^{238}U/^{235}U$ ratio in the extractable fraction (1 M ammonium acetate buffered at a pH of 4.5) of the DU-treated substrates showed noticeable deviations from the accepted natural isotopic ratio (137.88) and the isotopic ratio varied linearly with increasing dose of the DU spike. Similarly, the $^{238}U/^{235}U$ ratios in seedlings grown in DU-treated pots were consistent with the ratios determined in the extractable portion of the DU-treated substrates. The elevated isotopic ratio in the spruce seedlings suggests that the U taken up

is predominantly the dissolved depleted U. Consequently, metals taken up by vegetation are derived essentially from the more available cation pool in groundwater and/ or soil pore water. The study also revealed that the analysis of $^{238}U/^{235}U$ ratios in metal accumulator plants serves as important proxies for discriminating radionuclide contaminations in soil, groundwater, and perhaps the bedrock since plants can interact and absorb the metallic constituents of these media through the extensive root system.

Though the use of NAA and ICP-QMS techniques discriminated the presence of DU in the spiked substrates and in the stems of plants grown in the treated substrates, the precisions for the NAA measurements of isotopes ^{235}U and ^{238}U were substantially poor, hence not suitable for use in the current study. As demonstrated in the results, ICP-QMS analysis produced more précised isotope ratios and therefore more reliable technique. The ranges of RSDs for the $^{238}U/^{235}U$ ratio in DU-spiked substrates were between 0.06% and 2% and in stems of seedlings grown on them were between 0.1% and 1.3%. This infers that U taken up by spruce trees was predominantly from the labile species present in soil pore water and/or groundwater.

Appendix 6

[Refer to the attached data folder]

Appendix 6- 1: Element concentrations in the 7-10 year old black spruce twigs sampled from the Jacque's Lake area of the CMB, Labrador.

Appendix 6- 2: Element concentrations in black spruce bark collected from the Jacque's Lake area of the CMB, Labrador.

Appendix 6- 3: Element concentrations in Labrador tea stems sampled from the Jacque's Lake area of the CMB, Labrador.

Appendix 6- 4: Element concentrations in the less <250 μm sized-fraction of B-horizon soil from the Jacque's Lake area of the CMB, Labrador.

Appendix 6- 5: Element concentrations in Labrador tea leaves sampled from the Jacque's Lake area of the CMB, Labrador.